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To

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ABBREVIATIONS

AUC	= Area under the concentration curve
β -blocker	= β -Adrenoceptor blocking agent
$C_{\max}$	= Peak concentration
CMI	= Carbimazole
Con A	= Concanavalin A
cpm	= Counts per minute
Ig	= Immunoglobulin
LATS	= Long-acting thyroid stimulator
MIF	= Migration inhibition factor
MMI	= Methimazole
PHA	= Phytohemagglutinin
PTU	= Propylthiouracil
PWM	= Pokeweed mitogen
RIA	= Radioimmunoassay
T_3	= Triiodothyronine
T_4	= Thyroxine
$t_{1/2}$	= Elimination half-life
$t_{\max}$	= Time to reach peak concentration
TBII	= Thyrotropin binding inhibitor immunoglobulins
TSH	= Thyrotropin
TSI	= Thyroid stimulating immunoglobulins



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A. AIMS OF THE STUDY

The aims of the present study were to

- test the hypothesis that a defect in suppressor T lymphocyte function is involved in the pathogenesis of Graves' disease, by studying the suppressor T lymphocyte function,
- compare the *in vitro* effects on thyroid peroxidase activity of the thionamides carbimazole, methimazole and propylthiouracil and the β -adrenoceptor blocking agent propranolol,
- investigate whether methimazole and propylthiouracil have immunomodulatory effects, by studying their influence on mitogenic activation of lymphocytes *in vitro*,
- explore whether the β -adrenoceptor blocking agents propranolol (non-selective), metoprolol (cardioselective) and atenolol (cardioselective) have immunomodulatory effects, by studying their influence on mitogenic activation of lymphocytes *in vitro*,
- compare the kinetics of methimazole following the administration of carbimazole and methimazole respectively,
- explore if hyperthyroidism influences the kinetics of methimazole and β -adrenoceptor blocking agents (propranolol, metoprolol and atenolol).

B. BACKGROUND

THE IMMUNE SYSTEM AND GRAVES' DISEASE

Toxic diffuse goitre (Graves' disease) is the major type of hyperthyroidism (Werner and Ingbar 1978). It was originally described by Parry (1825) and later in more detail by Graves (1838) and von Basedow (1840). Apart from hyperthyroidism, specificophthalmologic and dermatologic changes (Werner and Ingbar 1978) are inherent manifestations of Graves' disease. The disease is probably genetically influenced (Werner 1978a). The exact pathogenesis is unknown but there is increasing evidence that the immune system is involved (Kidd et al. 1980). In addition, other pathogenetic mechanisms have been proposed (Werner 1978b), but the following discussion will be limited to the immune system.

Lymphocytes

The lymphocytes play a central role in the immune system. In man, they can be divided into several subpopulations, the two major categories being T (thymus derived) and B (bursa-equivalent derived) lymphocytes (Denman 1978). T lymphocytes are responsible for cell mediated immunoreactions and are involved in immunoregulatory functions (Bach and Bach 1981). B lymphocytes are precursors of immunoglobulin secreting plasma cells and responsible for humoral antibody production (Denman 1978). Lymphocytes can be classified according to their surface markers. The T lymphocytes are often defined by their receptors for sheep red blood cells, while the B lymphocytes generally are identified by their surface membrane immunoglobulins (Denman 1978).

The T lymphocytes are usually subdivided in two major subsets: helper T lymphocytes and suppressor T lymphocytes (Denman 1978). In the presence of antigens, the former cells help the B cells to mature into plasma cells and to form antibodies. Suppressor T cells have the opposite function and limit specific immune responses, *e.g.* by inhibiting the maturation of activated B cells and thus the release of antibodies (Denman 1978). This suppressive function may also inhibit the response to an autoantigen. A defect in this function could be important in the pathogenesis of autoimmune diseases (*vide infra*).

Mitogens and lymphocyte activation

Lymphocytes can be activated *in vitro* to transform and synthesize DNA by specific antigens but also by polyclonal activators *e.g.* the plant mitogens phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) (Weber 1969). The mitogens activate a majority of lymphocytes of a particular type in contrast to the very limited number of cells activated by a specific antigen (Rich and Pierce 1973). However, the sequence of events after activation of lymphocytes by a specific antigen or by a mitogen is similar (Rich and Pierce 1973). Therefore, mitogens have been extensively used to study the events of lymphocyte activation, *e.g.* in the evaluation of cell mediated immunity. In addition, the mitogens partially activate different populations of lymphocytes. PHA and Con A are mainly T cell mitogens, whereas PWM stimulates both T and B lymphocytes (Janossy and Greaves 1971, Rich and Pierce 1973).

The proliferative response of lymphocytes to mitogens can be assessed by the cellular uptake of radiolabelled thymidine, which reflects DNA synthesis (Polgar et al. 1968).

Humoral immunity

In 1956, Adams and Purves (1956) detected an abnormal thyroid stimulator in serum from patients with Graves' disease and this was the first clear evidence for an immunological disturbance in this malady. This thyroid stimulator, although sharing numerous biological characteristics with thyrotropin (TSH) (Kendall-Taylor 1975), is more long-acting as compared to TSH and for that reason was named "long-acting thyroid stimulator" (LATS) (Adams 1961). LATS was found to be an immunoglobulin (Kriss 1964) and could be detected in 50-70% of patients with untreated active Graves' disease (Kendall-Taylor 1975, Lamberg et al. 1969). The detection of LATS fostered the hypothesis that thyroid-stimulating immunoglobulins are responsible for the hyperfunction of the thyroid gland in Graves' disease (McKenzie 1959). However, no correlation was found between the titre of LATS and the clinical or laboratory degree of hyperthyroidism (Kendall-Taylor 1975).

In recent years, new techniques to measure immunoglobulins with thyroid-stimulating capacity (thyroid-stimulating immunoglobulins, TSI) were developed (Onaya 1973). New techniques also became available which measure antibodies binding to the TSH receptor (Manley et al. 1974, Smith and Hall 1974) and thus competing with the binding of TSH (thyrotropin binding inhibitor immunoglobulins, TBII). The TBII technique will occasionally detect blocking antibodies which bind and displace TSH but do not stimulate the thyroid gland (Endo et al. 1978). Using these techniques TSI can be detected in about 80% or more (Kuzuya et al. 1979, Sugeno et al. 1979, Zakarija et al. 1980) of patients with Graves' disease and similar figures have been found for TBII (Lamberg et al. 1979, O'Donnell et al. 1978, Teng and Yeung 1980). It seems clear that TSI and TBII are antibodies to the TSH receptor on the thyroid follicle cell (Kidd et al. 1980, Mäkinen et al. 1978).

In addition to the TSH receptor, other thyroid constituents may serve as antigens in man. Thus, antibodies have been detected against thyroglobulin (Roitt et al. 1956), microsomal antigen (Roitt et al. 1964), colloid components other than thyroglobulin (Doniach 1975), T_4 and T_3 (Staeheli et al. 1975). In contrast to that of TSI, the pathogenetic importance of these other thyroid antibodies is uncertain.

Cellular immunity

In peripheral blood from patients with Graves' disease there is often a relative lymphocytosis (Whartin 1928), and several studies have demonstrated a normal proportion of B and T lymphocytes (Lundell et al. 1976, Maciel et al. 1976, Mulaisho et al. 1975, Urbaniak et al. 1974, Wara et al. 1973, Volpé and Row 1975), even if there are some contradictory results (Grinblat 1979, Mori 1980). However, within the thyroid gland of patients with Graves' disease Tötterman (1978) demonstrated a relative enrichment of B lymphocytes. This is of special interest because B lymphocytes can produce TSI.

Cell mediated immunity is often tested by MIF (migration inhibition factor) test, and positive MIF tests have been reported with different thyroid antigens in Graves' disease (Lamki et al. 1973, Okita et al. 1980, Tötterman et al. 1977). This demonstrates that the T lymphocytes are sensitized against thyroid antigens in Graves' disease (Okita et al. 1980).

The crucial question then is: how do the sensitized lymphocytes arise? It has been suggested that there could be a virus induced thyroidal antigenic change (Allison et al. 1971) which could initiate the immune processes. A different view regarding the pathogenesis of autoimmune thyroid diseases is held by Volpé (1977). He postulated that Graves' disease and other organ specific autoimmune diseases could be due to genetically determined defects in immunoregulation, *i.e.* in suppressor T lymphocytes. This defect could permit the survival of the appropriate organ specific self-reactive "forbidden clone" of helper T lymphocytes arising by random mutation. By interacting with its complementary antigen (the TSH receptor) this clone is stimulated to perform helper functions; it establishes a localized cell-mediated immune response and also cooperates with appropriate self-directed B lymphocytes already present to produce humoral antibodies (TSI) (Volpé 1977).

Techniques for studying suppressor T lymphocytes

The theory of a defect in suppressor T lymphocytes in autoimmune diseases has made the study of suppressor cells in these disorders essential and several techniques to investigate suppressor lymphocytes have been devised. Of the five tests described below the first three are functional assays, whereas the others only estimate the number of suppressor T lymphocytes.

1. Activation of suppressor cells with Con A

This technique is based on the observation that incubation with Con A induces suppressor cell activity in lymphoid cells, which then can suppress the response to mitogens of autologous lymphocytes (Shou et al. 1976, Smith and Svejgaard 1981).

2. "Short-lived suppressor cell" test

This method is an application of the observation by Dutton (1972) that suppressor cells are short-lived or become functionally ineffective after incubation *in vitro* 24-48 h. Thus, the suppressor activity can be studied by comparing mitogenic activation of lymphocytes with and without preincubation (Bresnihan and Jasin 1977, Feighery et al. 1978). With this technique Bresnihan and Jasin

(1977) demonstrated a defect suppressor cell function in patients with systemic lupus erythematosus.

3. Sensitivity of suppressor cells to irradiation

It has been demonstrated that suppressor T cells are more radio-sensitive than helper T cells (Dutton 1972, Siegal and Siegal 1977). This can be used to demonstrate loss of suppressor cell activity after irradiation (Moretta et al. 1977, Siegal and Siegal 1977).

4. Presence of receptors for IgG on suppressor cells

According to Moretta et al. (1977) the helper T cells have surface receptors for the Fc part of IgM (T- μ) and the suppressor T cells for the Fc part of IgG (T- γ). Subdivision of T lymphocytes according to Fc receptors attracted great interest initially. Studies of blood from patients with autoimmune diseases indicated an abnormal distribution of T- μ and T- γ in these diseases (Moretta et al. 1977). As the Fc receptors are instable (Pichler et al. 1978) and as under some circumstances the T cells with IgM receptors have suppressor activity (Hayward et al. 1978, Pichler et al. 1978) these methods are less reliable (Editorial 1980, Pichler and Broder 1981).

5. Monoclonal antibody

Recently, T lymphocytes were classified by using monoclonal mouse antibodies directed at stable cell surface antigens (Reinherz and Schlossman 1980). Initial results with this technique indicate that the number of suppressor cells are decreased in patients with immuno-pathological disorders like systemic lupus erythematosus (Reinherz and Schlossman 1980) and in multiple sclerosis in relapse but not during remission (Bach et al. 1980, Reinherz et al. 1980).

THE PHARMACOLOGICAL MANAGEMENT OF GRAVES' DISEASE

Pharmacodynamics of drugs used in Graves' disease

The synthesis, secretion and metabolism of thyroid hormones can be affected by a variety of compounds (Green 1978) and several drugs have been employed in the treatment of hyperthyroidism (Green 1978). The thionamides represent the mainstay of pharmacotherapy of hyperthyroidism (Solomon 1978) and those registered in Sweden are propylthiouracil (PTU), methimazole (MMI) and carbimazole (CMI) (Almqvist and Melander 1980) (fig. 1). These agents inhibit thyroid hormone synthesis most probably by interference with the oxidation of iodide that is catalyzed by thyroid peroxidase (Taurog 1976). In addition, PTU can reduce the peripheral deiodination of thyroid hormones (Geffner et al. 1975), but the clinical importance of this is uncertain (Kampmann and Hansen 1981). The effect of CMI is at least partly mediated by conversion to MMI (fig. 1) (Lawson et al. 1951, Marchant et al. 1978, Nakashima and Taurog 1979) and it is unclear if CMI possesses any intrinsic antithyroid activity.

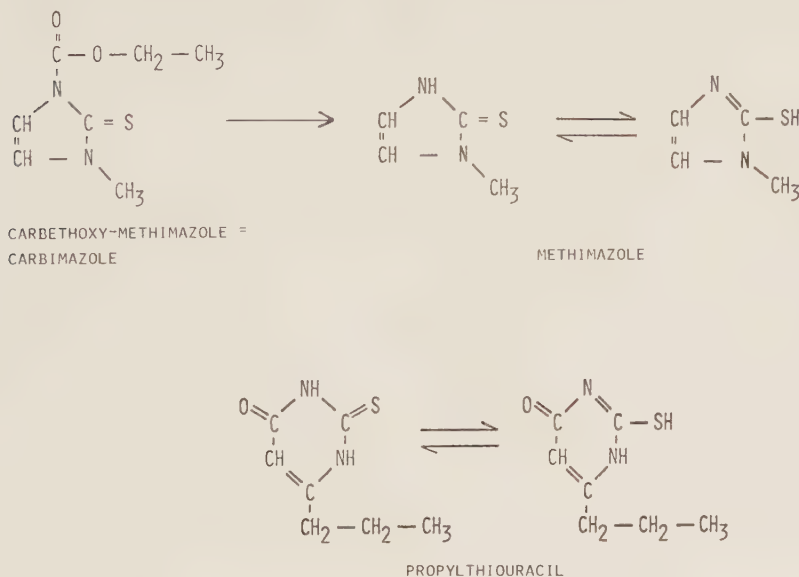


Fig. 1. Structures of the thionamides carbimazole, methimazole and propylthiouracil.

Assuming that Graves' disease is due to an immunological disturbance (Kidd et al. 1980) the ideal therapy should be directed against this disturbance. This kind of therapy is not available. However, it is possible that both surgery and radioiodine could influence an immunological process (Mukhtar et al. 1975) by reducing the thyroid cell mass and hence the amount of thyroid antigen. The commonly accepted basis for the use of thionamides is that they alleviate the hyperthyroid symptoms by inhibiting thyroid hormone synthesis, while the immunological disturbance - if present - is assumed to subside spontaneously (Marchant et al. 1978). However, it has been suggested that thionamides could have a direct immunomodulatory influence and thereby have an impact on the course of the disease (Beck et al. 1973, DeGroot 1977, Pinchera et al. 1969, Wall et al. 1976). In support of this concept, less lymphocytic infiltration of the thyroid was observed in patients with Graves' disease treated with CMI before operation, as compared to patients pretreated with propranolol (Beck et al. 1973). Furthermore, the LATS levels were reduced in patients treated with thionamides as compared to patients treated with radioiodine (Pinchera et al. 1969). Finally, PTU may exert an immunosuppressive effect as judged by its influence on *in vitro* mitogenic activation of peripheral blood lymphocytes from healthy subjects (Wall et al. 1976).

In addition to thionamides, β -adrenoceptor blocking agents (β -blockers) are commonly used in the treatment of Graves' disease. β -Blockers alleviate some of the symptoms of hyperthyroidism, probably by counteracting the sympathoadrenal component of the hyperthyroid state (Landsberg 1978). However, it seems possible that β -blockers, like thionamides, may have immunomodulatory properties. Such a possibility was suggested by animal studies demonstrating that propranolol enhances antibody production in mice (Nakazawa et al. 1976) and rats (Benner et al. 1968). Moreover, propranolol at high concentration inhibits mitogenic activation of human lymphocytes (Hadden et al. 1970).

Pharmacokinetics of drugs used in Graves' disease

A major cause of pharmacotherapeutic failures and side effects is the great interindividual variation in bioavailability, metabolism and excretion that exists for many drugs (Sjöqvist et al. 1976). As far as the treatment of hyperthyroidism is concerned, the situation is further complicated by the possibility that the hyperthyroid state and its subsequent normalization may alter the kinetics and effects of the drugs employed (Eichelbaum 1976). This may have important therapeutic implications.

As hyperthyroidism increases the general metabolic rate it would seem logical to suspect that the biotransformation of drugs in general would be enhanced in hyperthyroid patients. There are, however, rather few data to support this concept (Eichelbaum 1976) and it has even been contradicted for certain drugs (Gilette 1965). There are several reports (Balzer et al. 1975, Bell et al. 1977, Crooks et al. 1973, Feely et al. 1979, Kampmann and Skovsted 1975, McMurphy et al. 1975, Vesell et al. 1975) regarding the influence of hyperthyroidism on the kinetics of drugs specifically used in the treatment of this disorder, *i.e.* thionamides and β -blockers. However, sufficiently selective analytical techniques have become available only recently (Kampmann and Hansen 1981).

C. THE PRESENT STUDY

MATERIAL AND METHODS

a) Normal subjects and patients (I-V)

To explore suppressor T lymphocyte function in Graves' disease (I) studies were performed in one group of patients in the hyperthyroid state (5 males and 10 females; mean age 48 years; range 25-72 years) and in another group in the euthyroid state after treatment (3 males and 18 females; mean age 44 years; range 23-67 years). Clinically healthy laboratory staff members (9 males and 12 females; mean age 35 years; range 25-60 years) served as controls. Detailed data are given in I.

In the thyroid peroxidase activity studies (II), normal thyroid tissue was obtained from 3 euthyroid patients undergoing neck surgery for hyperparathyroidism.

In the studies of immunomodulatory effects of drugs (I, III, IV) fresh blood was obtained from healthy laboratory personnel (I, III, IV) or from healthy blood donors (III) and from 5 hyperthyroid patients (III, where detailed data of these patients are given).

Clinically healthy volunteers (3 males and 8 females; range 22-37 years) participated in the studies of MMI kinetics following the administration of CMI and MMI (II).

The kinetics of MMI and β -blockers were studied in patients with hyperthyroidism (7 males and 22 females; 18-74 years old) both during the hyperthyroid and euthyroid states; the MMI group included 8 patients and each β -blocker group 7 patients (detailed data are given in V).

b) Lymphocyte sampling and culture conditions (I, III, IV)

Lymphocytes were obtained from heparinized venous blood samples (I, III, IV) and from the buffy coat of fresh donor blood (III). The lymphocytes were prepared by centrifugation on Ficoll-Isopaque (Lymphoprep, Nyegaard and Co A/S, Oslo, Norway) as described by Bøyum (1968).

Lymphocyte samples were cultured at 37°C in microtitre plates (Linbro Scientific Inc, Hamden, CT, USA) containing 2.5×10^5 lymphocytes per culture in 200 μ l serum free RPMI 1640 (Gibco-Bio-Cult, Glasgow, Scotland) with the addition of gentamicin (12 μ g/ml), glutamine (2 mmol/l) and 1 or 5 g bovine serum albumin per liter (I, IV) or 10% heat inactivated pooled normal human serum (III).

When studying suppressor T lymphocyte function (I), the lymphocytes were incubated both in the absence and the presence of PWM (Sigma Chemical Co, St Louis, MO, USA; 1.25 μ g/ml). The PWM concentration was chosen to give suboptimal lymphocyte stimulation. PWM was added either at the beginning of culture (non-preincubated culture) or after 24 h of culture (preincubated culture). All cultures were carried out in triplicate. The cells were cultured for 1-7 days

and at each 24 h period a fraction of the cultures was harvested and the DNA synthesis was measured (*vide infra*).

In the studies of immunomodulatory effects of drugs (I, III, IV) the lymphocytes were incubated for 72 h in the presence of PHA (Wellcome Ltd, London, England; 1.25 µg/ml), Con A (Pharmacia Fine Chemicals, Uppsala, Sweden; 5.0 µg/ml; in I and III) or PWM 2.5 µg/ml. These concentrations were chosen to give optimal lymphocyte stimulation. Drugs were dissolved in RPMI 1640 and added to the cultures either simultaneously with the mitogens at the beginning (I, III, IV) or after 47.5 h (III) to identical parallel cultures. All cultures were carried out in triplicate except for controls in I and IV, which were quintuples. After 72 h the cells were harvested and the DNA synthesis was measured (*vide infra*).

c) DNA synthesis (I, III, IV)

[³H] Thymidine (0.5 µCi; NET-027Z; specific activity 48 Ci/mmol; New England Nuclear Corp, Boston, MA, USA) in RPMI 1640 was added 22-24 h before harvesting the lymphocytes. The cells were harvested on to glass fibre filters and rinsed in distilled water using a Skatron harvesting machine (Lierbyen, Oslo, Norway). The filters were dried 30 min under a heat lamp and transferred to scintillation vials containing 4 ml Insta-Fluor solution (Packard, Downers Grove, IL, USA). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter and expressed in counts per minute (cpm).

d) Peroxidase activity (II)

Thyroid tissue specimens were homogenized as described in II. Peroxidase activity in thyroid tissue was assayed by a method of Lundquist and Josefsson (1971) employing a reagent solution containing o-dianisidine, glucose oxidase, Triton-X-100, citric acid and sodium hydroxide, which was added to a mixture of glucose, ammonium hydroxide and calcium chloride when the incubation was started. The drugs were dissolved in saline and then added to the medium together with the glucose-ammonia-calcium mixture. Particular care was taken to maintain the pH at 6.0 in order to avoid any unintentional conversion of carbimazole to methimazole as may readily occur in plasma and other alkaline media (Lawson et al. 1951, Nakashima and Taurog 1979). Previous experiments

had demonstrated that in the system used the pH optimum for peroxidase activity in human thyroid was between 6.0 and 6.5. Incubation was carried out at 37°C for 30 min.

e) Drugs and their assays (I-V)

For the *in vitro* studies the drugs used were obtained directly from the manufacturers. In the *in vivo* studies commercially available drugs were employed. The following drugs were used: T₃ (I; British Drug House, Poole, England), CMI (II; Neo-Mercazole^R; Nicholas Laboratories Ltd, Slough, England), MMI (II, III, V; Thacapzol^R; AB Kabi, Stockholm, Sweden), 6-PTU (II, III; AB Pharmacia, Uppsala, Sweden), D-, L-propranolol (II, IV, V; Inderal^R; ICI Pharmaceuticals, Macclesfield, England), D-propranolol and L-propranolol (IV; ICI Pharmaceuticals), atenolol (IV, V; Tenormin^R, ICI Pharmaceuticals), metoprolol (IV, V; Seloken^R; AB Hässle, Mölndal, Sweden), quinidine and procaine (IV; AB Hässle) and lidocaine (IV; AB Astra, Södertälje, Sweden).

T₃ in study I was tested in a final concentration of 0.1, 1, 10, 100 and 1000 nmol/l. In the thyroid peroxidase activity studies (II) the drug concentrations were 100, 330 and 1000 µmol/l. In study III, PTU and MMI were tested in a final concentration of 5, 50, 500 and 1000 µmol/l except for MMI tested at 1, 10, 100 and 1000 µmol/l when using PHA as mitogen. All drugs in study IV were tested in a final concentration of 0.01, 0.1, 1, 10, 100 and 1000 µmol/l.

The concentrations of MMI (II, III, V) were determined by high-pressure liquid chromatography as described by Skellern et al. (1974, 1976) with some minor modifications (details given in II). The MMI assay was always carried out within 24 h after blood collection (II, V). The PTU concentrations in incubation media (III) were determined by a modification of the MMI method (details given in III). Propranolol (IV, V) and atenolol (IV, V) concentrations were determined by high-pressure liquid chromatography (Pritchard et al. 1979, Scales and Copsey 1975, Yee et al. 1979), metoprolol by high-pressure liquid chromatography (IV) (Scales and Copsey 1975, Yee et al. 1979) or by gas chromatography (V) (Ervik 1975).

f) Design of the pharmacokinetic studies (II, V)

In study II each subject came to the laboratory twice with an interval of at least 7 days. On both occasions they arrived in the morning after an over-night fast. They were then given 60 mg (12 tablets $\hat{a}$ 5 mg) of CMI or of MMI in a randomized manner. The tablets were taken immediately after the intake of a standardized breakfast of 1840 kJ (Melander 1978). Blood samples for analyses of the drug concentrations in serum were taken before the intake of the meal and the respective drug and then repeatedly for 24 h.

In study V each patient fasted over-night and then received a single oral dose of either 40 mg MMI, 80 mg propranolol, 100 mg metoprolol or 100 mg atenolol. A regular hospital breakfast was consumed 2 h after the administration of MMI, whereas the β -blockers were given immediately before a standardized breakfast of 1840 kJ (Melander 1978). The same drugs were administered once more to the same patients in an identical manner, after clinical and biochemical euthyroidism had been present for at least 4 weeks, following treatment either with drugs alone, or surgery after pretreatment with β -blockers, or radioiodine or radioiodine combined with thionamides. Blood samples for the determination of plasma drug concentrations were taken before and repeatedly after the administration of the drug.

g) Calculations and statistical evaluation (I-V)

When studying suppressor T lymphocyte function (I) the median cpm value of each triplicate minus the median cpm value for corresponding culture without mitogen was calculated. The value thus obtained from the day of maximum response for each culture was used to estimate the suppressor lymphocyte function by the quotient preincubated culture value/non-preincubated culture value.

In the studies of immunomodulatory effects of drugs (I, III, IV) the median value of each triplicate was calculated as the percentage of control, *i.e.* the value derived from culture with mitogen in the absence of drugs.

In paper V the elimination half-life of each drug ($t_{1/2}$) was calculated by regression analysis. The area under the concentration curve (AUC) was determined by the trapezoidal rule. The residual ($8\text{ h} - \infty$) AUC values ("rest areas") of the β -blockers were not included in the AUC calculations, as they were rather great and hence difficult to estimate accurately. Peak concentration ($C_{\max}$) was defined as the highest value actually recorded and the time to reach peak concentration ($t_{\max}$) was obtained accordingly. As none of the three β -blockers has 100% oral bioavailability (Johnsson and Regårdh 1976) and as the oral bioavailability of MMI is unknown, calculation of absorption and distribution kinetics was not possible.

The statistical significance of difference was assessed by Student's t -test for unpaired (I, II in pharmacodynamic studies, III, IV) or paired observations (II in pharmacokinetic studies). In paper I, Wilcoxon's rank sum test for unpaired (suppressor study) and paired (T_3 study) samples was used besides Student's t -test. In paper V, Wilcoxon's rank sum test for paired samples was used to determine intraindividual differences. The correlation coefficient (r) was calculated by conventional methods (I). The level of significance was taken as $p < 0.05$. Values in I (suppressor study) and in figs 2-5 are given as mean $\pm$ SEM. * Denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

RESULTS

1. Suppressor T lymphocyte function in Graves' disease (I)

The quotient preincubated culture value/non-preincubated culture value was used to estimate the suppressor T lymphocyte function. The quotient for the hyperthyroid group (1.00 ± 0.07) differed ($p < 0.01$) from that of the control group (1.37 ± 0.08) but not from the quotient of the euthyroid patient group (1.12 ± 0.05). Also the quotients for the euthyroid patient group and the control group differed ($p < 0.05$). There was no significant correlation between the recorded quotients and the serum T_3 values for the hyperthyroid group ($r = 0.027$).

2. Pharmacodynamic studies

Influence of thionamides and propranolol on thyroid peroxidase activity (II)

MMI exerted a strong inhibitory influence on peroxidase activity giving a significant ($p < 0.001$) reduction at each concentration. On an equimolar basis PTU had a weaker effect, which was significant at the highest ($p < 0.01$) and the intermediate ($p < 0.05$) but not at the lowest concentration. CMI gave a slight inhibition ($p < 0.05$) but only at the highest concentration. Propranolol caused no significant inhibition at any concentration studied.

Influence of thionamides, β -adrenoceptor blocking agents and triiodothyronine on mitogenic activation of lymphocytes

Thionamides (III). In studies, where drug and mitogen were added concomitantly, at $1000 \mu\text{mol/l}$, but not at the other tested concentrations, PTU slightly reduced ($p < 0.02$), whereas MMI augmented ($p < 0.02$) PHA-induced accumulation of [^3H] thymidine in peripheral blood lymphocytes from healthy subjects (fig. 2).

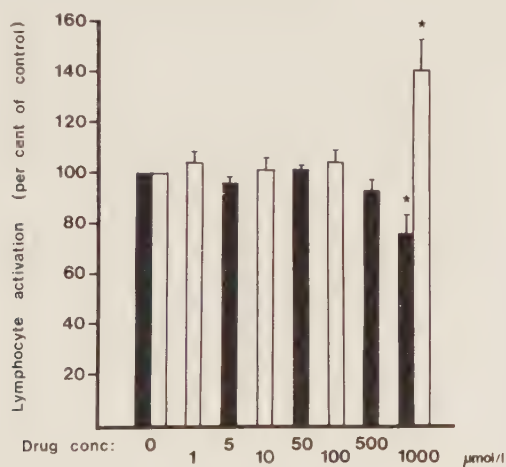


Fig. 2. Uptake of [^3H] thymidine in human peripheral blood lymphocytes activated with PHA in the presence and absence of PTU (filled columns) or MMI (open columns), $n = 7$.

PTU had similar effects when using peripheral blood lymphocytes from hyperthyroid patients; at 1000 $\mu\text{mol/l}$ PTU evoked a 23% reduction ($p < 0.001$). MMI was not tested against lymphocytes from hyperthyroid patients. With Con A as mitogen, at 1000 $\mu\text{mol/l}$, but not at the other tested concentrations, PTU ($n = 6$) reduced ($p < 0.02$), whereas MMI ($n = 5$) augmented ($p < 0.05$) the accumulation of [^3H] thymidine in peripheral blood lymphocytes from healthy individuals. Both PTU and MMI had a stimulatory effect on PWM-activation of peripheral blood lymphocytes from healthy subjects (fig. 3). Thus, PTU significantly enhanced [^3H] thymidine accumulation at 5 ($p < 0.02$), 50 ($p < 0.005$) and 500 $\mu\text{mol/l}$ ($p < 0.02$) and MMI at 500 ($p < 0.01$) and 1000 $\mu\text{mol/l}$ ($p < 0.005$).

When PTU or MMI was added after 47.5 h of incubation the effects on mitogenic activation of peripheral blood lymphocytes were similar to those recorded with concomitant addition of drug and mitogen.

In a special experiment, PTU and MMI concentrations in the medium were quantified repeatedly throughout the experiment at all concentrations used. The results demonstrated that the intended concentrations were present and maintained throughout the incubation time.

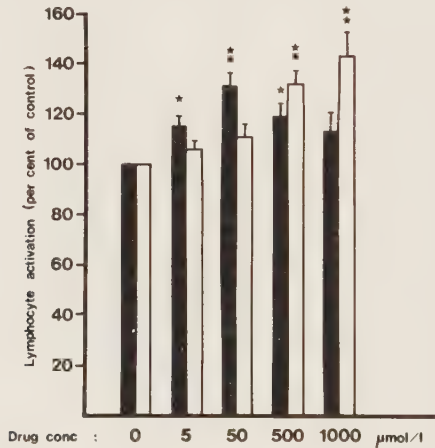


Fig. 3. Uptake of [^3H] thymidine in human peripheral blood lymphocytes activated with PWM in the presence and absence of PTU (filled columns; $n = 6$) or MMI (open columns; $n = 5$).

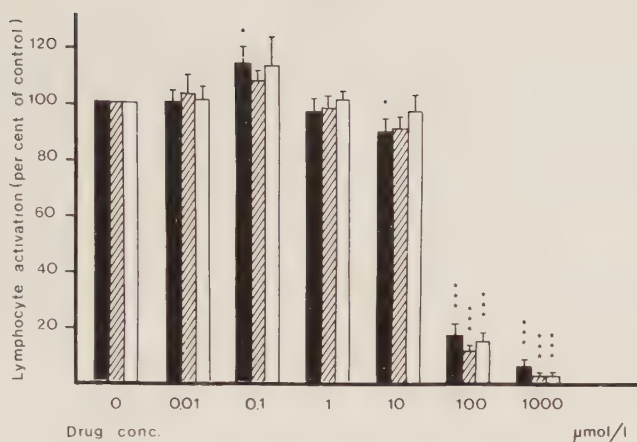


Fig. 4. Uptake of [3 H] thymidine in human peripheral blood lymphocytes activated with PHA in the presence and absence of L-propranolol (filled columns), D-, L-propranolol (hatched columns) or D-propranolol (open columns), $n = 7$.

β -Blockers (IV). D-propranolol, L-propranolol and D-, L-propranolol at 100 and 1000 $\mu\text{mol/l}$ strongly reduced ($p < 0.001$) PHA-induced accumulation of [3 H] thymidine in peripheral blood lymphocytes (fig. 4). With L-propranolol a minor reduction was seen at 10 $\mu\text{mol/l}$ ($p < 0.05$), whereas a slight augmentation was recorded at 0.1 $\mu\text{mol/l}$ ($p < 0.05$). Neither D- nor D-, L-propranolol had significant effects at these concentrations. In a small series ($n = 3$) of experiments using drug concentrations between 10 and 100 $\mu\text{mol/l}$, D-propranolol evoked a mean reduction of 37%, L-propranolol 25% and D-, L-propranolol 39% at 25 $\mu\text{mol/l}$. The corresponding mean reductions at 50 $\mu\text{mol/l}$ were 55%, 44% and 55%, respectively. With PWM as mitogen, D-, L-propranolol ($n = 8$) gave a strong reduction ($p < 0.001$) at 100 and 1000 $\mu\text{mol/l}$; no significant effects were recorded at the other tested concentrations.

Metoprolol ($n = 7$) at 1000 $\mu\text{mol/l}$ slightly reduced ($p < 0.05$) and at 1 $\mu\text{mol/l}$ slightly augmented ($p < 0.02$) PHA-induced accumulation of [3 H] thymidine in peripheral blood lymphocytes. Atenolol ($n = 7$) at 1 $\mu\text{mol/l}$ slightly augmented ($p < 0.02$) PHA-induced [3 H] thymidine incorporation. Both metoprolol and atenolol were without significant effects at the other tested concentrations. With PWM as mitogen atenolol ($n = 9$) had no significant effect.

Lidocaine ($n = 7$) at $100 \mu\text{mol/l}$ but not at the other tested concentrations slightly reduced ($p < 0.05$) [^3H] thymidine incorporation in peripheral blood lymphocytes. Procaine ($n = 5$) had no significant effect. Quinidine ($n = 5$) at 100 and $1000 \mu\text{mol/l}$ strongly reduced ($p < 0.001$) PHA-induced accumulation of [^3H] thymidine and at $10 \mu\text{mol/l}$ evoked a 23% reduction ($p < 0.05$).

After 72 h of culture the following results of cell viability were found: RPMI without drug 91%, with metoprolol $1000 \mu\text{mol/l}$ 90%, D-propranolol $1000 \mu\text{mol/l}$ 0%, $100 \mu\text{mol/l}$ 91%, L-propranolol $1000 \mu\text{mol/l}$ 1%, $100 \mu\text{mol/l}$ 85%, D-, L-propranolol $1000 \mu\text{mol/l}$ 1%, $100 \mu\text{mol/l}$ 91%.

Measurements of the concentrations of propranolol, metoprolol and atenolol in the incubation media before and after 72 h incubation revealed no degradation of either β -blocker.

T_3 (I). T_3 at 1000 nmol/l but not at the other tested concentrations slightly reduced ($p < 0.05$) PWM-induced ($n = 10$) accumulation of [^3H] thymidine in peripheral blood lymphocytes and had no influence on PHA- ($n = 10$) or Con A- ($n = 7$) activation of peripheral blood lymphocytes.

3. Pharmacokinetic studies

Methimazole kinetics following the administration of carbimazole and methimazole (II). After the administration of equal amounts of CMI and MMI there were great interindividual differences in C_{max} , $t_{1/2}$ and AUC values of serum MMI. Within individuals there was no significant difference in $t_{1/2}$ or t_{max} . However, both the C_{max} and the AUC values were greater ($p < 0.01$) following the administration of MMI itself than after CMI.

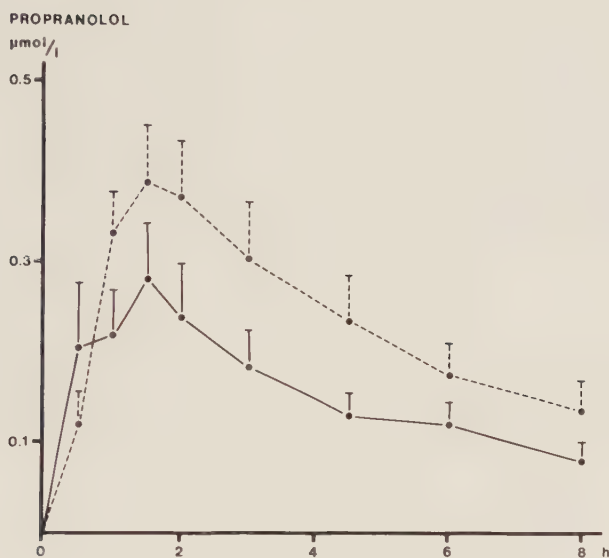


Fig. 5. Kinetic profile of orally administered propranolol in patients (n = 7) during the hyper- (—) and euthyroid (---) states.

Influence of hyperthyroidism on the kinetics of methimazole and β -adrenoceptor blocking agents (V). For MMI neither the C_{max} , the t_{max} , the $t_{1/2}$ nor the AUC (0 h - ∞) values were affected by the hyperthyroid state. For propranolol (fig. 5) and metoprolol on the other hand the AUC (0-8 h) values were lower ($p < 0.02$) during the hyperthyroid state than during the euthyroid state. As the t_{max} for propranolol and metoprolol were shortened rather than prolonged in the hyperthyroid state, the cut-off of the AUC at 8 h would rather under- than overestimate the two differences. Hence, the calculated AUC difference most probably reflects a true difference. The C_{max} and the $t_{1/2}$ for propranolol and metoprolol were not significantly altered during the hyper- and euthyroid states.

In contrast to those of propranolol and metoprolol, the AUC (0-8 h) values of atenolol were not significantly different during the hyperthyroid as compared to the euthyroid state. Neither the C_{max} , the t_{max} nor the $t_{1/2}$ values were significantly influenced by the hyperthyroid state.

DISCUSSION

1. Suppressor T lymphocyte function in Graves' disease

Volpé (1977) suggested that Graves' disease and other autoimmune diseases may be due to impaired immunoregulation possibly related to a defect in suppressor T lymphocyte function. To test this hypothesis different methods evaluating the number (Canonica et al. 1981) or the function (Aoki et al. 1979, Balázs et al. 1979, Okita et al. 1981) of suppressor T lymphocytes have been used. Since a normal number of suppressor T lymphocytes does not exclude functional defects (Editorial 1980, Waldmann 1978) we considered it essential to use a functional test. The "short-lived suppressor cell" test was chosen in the present study as it is a well established functional test (Bresnihan and Jasin 1977, Feighery et al. 1978, Schuster et al. 1979) as documented also in Graves' disease (Aoki et al. 1979, Balázs et al. 1979).

Our results in the hyperthyroid group agree with those of Aoki et al. (1979) and Balázs et al. (1979) using the same technique. This supports the concept of a defect in suppressor T lymphocyte function in Graves' disease. However, the recorded effects might be due to the hyperthyroid state *per se*. For this reason, our study was extended to include also euthyroid patients previously treated for Graves' disease. Also in this group the quotient used to estimate suppressor T lymphocyte function was significantly different from that of the control group, but there was a tendency to normalization. This tendency may reflect heterogeneity among the euthyroid patients previously treated for Graves' disease (Kidd et al. 1980); some could have undergone a true immunological remission, while others retained an altered immunity. Anyhow, the results recorded in the euthyroid patient group indicate that the findings in the hyperthyroid group were not mainly due to the hyperthyroid state *per se*. This view was further supported by the lack of correlation between the serum T_3 values and the quotients in the hyperthyroid group.

Recently, Okita et al. (1981) using a modified MIF test, demonstrated decreased suppressor T lymphocyte activity in hyperthyroid patients with Graves' disease. In addition, Canonica et al. (1981) reported a reduced number of T cells bearing receptors for the Fc portion of IgG, and Sridama et al. (1981) found a relative decrease of suppressor T cells in hyperthyroid Graves' disease as tested with monoclonal antibodies. Thus, the results of the present and several other

studies using different techniques are consistent with the hypothesis of a defect in suppressor T lymphocyte function in hyperthyroid patients with Graves' disease. The present study further indicates that this defect may persist in the euthyroid state after treatment. This is in accordance with a recent report of How et al. (1981) demonstrating a positive MIF test as well as a defect in suppressor T lymphocyte function in 16 of 25 euthyroid patients previously treated for Graves' disease.

2. Pharmacodynamic studies

Influence of thionamides and propranolol on thyroid peroxidase activity

The precise mechanism for the therapeutic effect(s) of thionamides in Graves' disease is not clear. However, there is evidence that one mode of action is interference with thyroid peroxidase activity (Taurog 1976), which leads to a reduction of thyroid hormone synthesis. The present findings support this view and show that MMI is a stronger peroxidase inhibitor than PTU (Davidson et al. 1978, Taurog 1976).

It is known that CMI is rapidly converted to MMI and that its effect is at least partially dependent on this conversion (Lawson et al. 1951, Marchant et al. 1978, Nakashima and Taurog 1979). Indeed, in the present study CMI as such had only a slight inhibitory effect on peroxidase activity and besides only at the highest concentration tested. Moreover, even though the incubations were carried out at pH 6, some conversion of CMI to MMI might still have occurred. Thus, the results suggest that CMI essentially is inactive *per se* and functions only as a pro-drug. Our finding that the β -blocker propranolol had no inhibitory influence on thyroid peroxidase activity is in agreement with the concept that propranolol does not affect thyroxine biosynthesis (Wartofsky et al. 1975).

Influence of thionamides, β -adrenoceptor blocking agents and triiodothyronine on mitogenic activation of lymphocytes

Thionamides. In the present study, PTU significantly suppressed PHA- and Con A-induced activation of lymphocytes at a PTU concentration of 1000 $\mu\text{mol/l}$ but not at lower concentrations. This is in agreement with the recent report of Finke et al. (1981) that PTU has an inhibitory influence on Con A activation only at high PTU concentrations (500 $\mu\text{mol/l}$) but in contrast to the finding of Wall et al. (1976) that PTU exerts a suppressive effect at 5.88 $\mu\text{mol/l}$ (1 mg/l). Wall et al. (1976) reported that PTU strongly suppressed PWM induced activation, whereas we found slightly enhanced PWM activation. There is no obvious explanation for these discrepancies. However, in the present study, 6-propyl-thiouracil was used, whereas Wall et al. (1976) seem to have used 5-propyl-thiouracil. In addition, differences in the mitogen concentrations might have played a role. The possibility that degradation of the drug might have been of importance was excluded as the intended drug concentrations were found to remain constant throughout the incubations.

Our finding that MMI, in contrast to PTU, enhanced PHA- and Con A-induced activation indicates that the two drugs may be qualitatively different in their immunomodulatory capacity. On the other hand, PTU and MMI had similar enhancing effects on PWM induced activation.

The present study demonstrates that PTU and MMI interfere with mitogenic activation of lymphocytes *in vitro*. The effects were, however, rather small and occurred mainly at concentrations exceeding those obtained in blood after *in vivo* administration of these drugs (Sitar and Hunninghake 1975, cf paper II). Thus, caution is warranted as to the clinical importance of these findings. On the other hand, experiments in animals and humans indicate that thionamides accumulate in thyroid tissue (Jansson et al. 1981a, Lazarus et al. 1975, Marchant et al. 1972) and it is possible, therefore, that drug concentrations similar to those needed to affect mitogenic activation of lymphocytes may be attained within thyroid tissue during treatment of hyperthyroidism. Moreover, effect comparisons of *in vitro* and *in vivo* concentrations may not be valid (Segal and Ingbar 1980), as sensitivity could differ because of the *in vivo* presence of other factors that can enhance the response to a given agent (Segal and Ingbar 1980).

There are several *in vitro* and *in vivo* studies, particularly employing MMI/CMI, indicating that thionamides may have immunomodulatory effects and thus might influence the course of Graves' disease. Increasing concentrations of MMI (10 $\mu\text{mol/l}$ and higher) caused progressive inhibition of PWM induced thyroid autoantibody synthesis by cultured lymphocytes from patients with Hashimoto's thyroiditis (McGregor et al. 1980b). Inhibition of autoantibody synthesis was paralleled by a decrease in total IgG production. These observations may seem to contradict our findings of a stimulatory effect of MMI on mitogenic lymphocyte activation. However, the different results might relate to the fact that, in the cited study autoantibody synthesis and thus IgG production was measured, while we measured the uptake of [^3H] thymidine and thus the blastogenic response (Nespoli et al. 1980). McGregor et al. (1981) studied the influence of MMI on thyroglobulin-induced stimulation of lymphocytes from patients with Hashimoto's thyroiditis and in accordance with our results found that MMI at 100 $\mu\text{mol/l}$ had no influence on the blastogenic response to thyroglobulin but inhibited the thyroglobulin-driven immunoglobulin synthesis. Both PTU and MMI were recently reported to inhibit immunoglobulin production *in vitro* (Weiss and Davies 1981). Animal studies demonstrated that MMI can reduce both induction of thyroiditis and the severity of the disease once established (Keast et al. 1981). It has previously been reported that TSI/TBII levels fall in hyperthyroid patients when treated with thionamides (Bech and Madsen 1980, Fenzi et al. 1979, McGregor et al. 1979), and McGregor et al. (1980b) demonstrated that this fall was more pronounced in patients treated with CMI than with propranolol or placebo. In addition, thyroid microsomal antibody levels fell in patients with Hashimoto's thyroiditis during treatment with CMI (McGregor et al. 1980a).

β -Adrenoceptor blocking agents. The present study indicates that, at the lower tested concentrations, both L-propranolol, metoprolol and atenolol may increase PHA activation of lymphocytes. However, these effects were very slight and hence of doubtful clinical relevance.

The inhibitory effect of propranolol at the higher tested concentrations agrees with a previous report by Hadden et al. (1970). To investigate further the mechanism of this influence the effects of quinidine and two other membrane-stabilizing agents were examined besides the β -blockers. At 100 $\mu\text{mol/l}$,

D-propranolol and quinidine seemed as effective as L-propranolol in the present study. In contrast to L-propranolol, D-propranolol is virtually devoid of β -adrenoceptor blocking capacity, but both isomers have similar membrane-stabilizing or quinidine-like properties (Waal-Manning 1976). The present findings indicate that the recorded inhibitory effect of propranolol may be due to a membrane-stabilizing effect rather than to genuine β -adrenoceptor blockade. This view is further supported by the observation that the cardio-selective β -blocker metoprolol, which has very weak membrane-stabilizing properties (Waal-Manning 1976), exhibited only a slight suppressive effect at the highest dose (1000 $\mu\text{mol/l}$). Similarly, the cardioselective β -blocker atenolol, which is devoid of membrane-stabilizing properties (Waal-Manning 1976), had no suppressive influence at any concentration used. It is of interest that D-propranolol, like D-, L-propranolol, was capable of inhibiting conversion of T_4 to T_3 , again indicating an effect due to membrane stabilization and not to β -adrenoceptor blockade (Heyma et al. 1980).

McGregor et al. (1980b) recently reported that propranolol in concentrations greater than 10 $\mu\text{mol/l}$ inhibited thyroid autoantibody synthesis *in vitro*, but propranolol treatment had no effect on TBII or microsomal antibody titres *in vivo*. As for the clinical significance of the present *in vitro* findings it may be noted that treatment of hyperthyroid patients with 320 mg of propranolol *per diem* results in drug concentrations in plasma less than 1 $\mu\text{mol/l}$ (Nilsson et al. 1980). The inhibitory effect of propranolol on *in vitro* lymphocyte function was seen only at higher concentrations but as discussed above it may not be valid to compare effects of equimolar *in vitro* and *in vivo* concentrations (Segal and Ingbar 1980). As judged from the present study β -blockers do not seem to have immunomodulatory effects related to β -adrenoceptor blockade. However, propranolol might have such effects due to its membrane-stabilizing properties.

Triiodothyronine. When lymphocytes were activated with different mitogens in the presence of T_3 we found a slight suppressive effect on PWM activation but only at a supraphysiological concentration of T_3 (1000 nmol/l). Thus, our *in vitro* studies do not indicate that T_3 has immunomodulatory effects as suggested by Balázs et al. (1980). The reason for the discrepancy between our results and those of Balázs et al. (1980) is unknown.

3. Pharmacokinetic studies

Methimazole kinetics following the administration of carbimazole and methimazole

Following the oral administration of CMI the drug has never been recovered in its unchanged form in serum, urine or thyroid tissue (Kampmann and Hansen 1981, Skellern et al. 1974 and 1980). This is apparently due to a very rapid esterase-mediated hydrolysis of CMI to MMI, which is assumed to occur both in the first passage through gut and liver and in serum (Kampmann and Hansen 1981, Nakashima and Taurog 1979, Skellern et al. 1974 and 1980). It has been suggested that slow hydrolysis of CMI to MMI would promote a sustained release of MMI and thus prolong the effect (Pulver and Bründler 1957, Vanderlaan and Storrie 1955). However, in the present study neither the $t_{\max}$ nor the $t_{1/2}$ of MMI demonstrated any significant intraindividual difference after the administration of CMI and MMI, respectively. Similar observations were reported by Skellern et al. (1980). Hence, there is no support for a sustained release of CMI. Following the administration of equal amounts of MMI and CMI, both the $C_{\max}$ and the AUC values of MMI were greater (79% and 67%, respectively) after the intake of MMI. Presumably, this is related to the difference in molecular weight between CMI (186) and MMI (114), due to the presence of a carbethoxy side chain in CMI which is split off, when CMI is converted to MMI. Thus, employing the same amounts (in mg) of the two compounds, treatment with MMI would give higher plasma concentrations of MMI than treatment with CMI. This may be the reason for the higher frequency of adverse reactions occurring after MMI as compared to CMI, which reactions may be dose related (McGavack and Chevalley 1954, Wiberg and Nuttall 1972).

The findings in the present study support the view that CMI offers no therapeutic advantage over MMI, either from a pharmacodynamic or from a pharmacokinetic point of view.

Influence of hyperthyroidism on the kinetics of methimazole and β -adrenoceptor blocking agents

Our finding of unaffected MMI kinetics in hyperthyroidism does not support the view that hyperthyroidism would enhance drug metabolism in general. This is in accordance with a recent report of Jansson et al. (1981b) but in contrast to previous studies (Crooks et al. 1973, Vesell et al. 1975) reporting an altered

handling of MMI in hyperthyroidism. However, the conclusions in the latter studies were based on observations made with less specific methods (Skellern et al. 1980).

Propranolol and metoprolol undergo extensive presystemic clearance, probably by similar biochemical processes (Johnsson and Regårdh 1976). Hence it seems likely that the reduced AUC values of propranolol and metoprolol during the hyperthyroid state seen in the present study resulted from an enhanced presystemic clearance of these two β -blockers. This is a novel observation as to metoprolol, whereas similar findings have been reported for propranolol by Feely et al. (1981) and recently by Aro et al. (1982). Moreover, Riddell et al. (1980) and Rubenfeld et al. (1978) demonstrated that in the hyperthyroid state the systemic clearance of propranolol is increased following its intravenous administration. Thus, it seems likely that hyperthyroidism may enhance both the pre-systemic and the systemic clearance of propranolol and metoprolol. An altered distribution volume - *e.g.* caused by reduced protein binding - may contribute to this (Feely et al. 1981, Rubenfeld et al. 1978). Hyperthyroidism has been found to affect the distribution volume of some other drugs, such as PTU (Sitar et al. 1979) and paracetamol (Forfar et al. 1980).

In contrast to the present findings, Theilade et al. (1980) reported an increased AUC value for propranolol in hyperthyroid patients as compared to healthy volunteers. There is no obvious explanation for this discrepancy but it may relate to the fact that the data reported of Theilade et al. (1980) were derived from a between-subject comparison of hyperthyroid patients with healthy volunteers and not from within-patient comparisons as in the present study.

In further consonance with the concept that hyperthyroidism would enhance the presystemic clearance of propranolol and metoprolol it was found that hyperthyroidism affected neither the AUC nor the $t_{\max}$ of atenolol, which essentially escapes metabolic transformation (Johnsson and Regårdh 1976). In addition, Aro et al. (1982) reported that the kinetics of the β -blocker sotalol, which is not metabolized, was unaffected by the hyperthyroid state.

The present results indicate that there could be a pharmacokinetic basis for dose adjustments of propranolol and metoprolol but not of MMI and atenolol during the course of hyperthyroidism. It should be emphasized, however, that the pharmacodynamics of all four drugs may be influenced by the change in thyroid activity and that dose adjustments may be warranted for that reason.

D. GENERAL SUMMARY AND CONCLUSIONS

In order to test the hypothesis that a defect in suppressor T lymphocyte function is involved in the pathogenesis of Graves' disease, this function was studied in one group of patients in the hyperthyroid state and in another group in the euthyroid state after treatment. The suppressor T lymphocyte function was assessed by comparing mitogenic activation of peripheral blood lymphocytes with and without preincubation. A reduced function was recorded both in the hyperthyroid and in the euthyroid state.

The pharmacodynamics of two different types of drugs used in the management of Graves' disease *i.e.* thionamides and β -blockers were studied with respect to their *in vitro* effects on thyroid peroxidase activity and on mitogenic activation of lymphocytes. On an equimolar basis methimazole (MMI) exerted a strong inhibitory influence on peroxidase activity, while propylthiouracil (PTU) had a much weaker influence. Carbimazole (CMI) had a slight effect and only at the highest concentration (1000 $\mu\text{mol/l}$), and propranolol exerted no measurable peroxidase inhibition at any concentration. As for mitogenic activation of lymphocytes, PTU (at 1000 $\mu\text{mol/l}$) slightly suppressed PHA- and Con A-activation but enhanced (at 5-500 $\mu\text{mol/l}$) PWM-activation. MMI (500-1000 $\mu\text{mol/l}$) enhanced PWM-activation and (at 1000 $\mu\text{mol/l}$) also PHA- and Con A-activation of lymphocytes. Both racemic propranolol and its separate D- and L-isomers reduced PHA-activation of lymphocytes at 100 and 1000 $\mu\text{mol/l}$. A similar effect was obtained with quinidine. Even at 1000 $\mu\text{mol/l}$ metoprolol had only a slight suppressive effect on PHA-activation and atenolol had no suppressive influence at all.

Pharmacokinetic studies of MMI and β -blockers were also performed. When the kinetics of MMI was compared after oral administration of CMI and of MMI itself, there was no measurable difference in the time to reach peak concentration or in the half-life of MMI, but the peak concentrations and the AUC values of MMI were greater after the administration of MMI itself than after CMI. The kinetic profiles of orally administered MMI, propranolol, metoprolol and atenolol were compared in hyperthyroid patients both during the hyper- and the euthyroid state. For MMI and atenolol the kinetic profiles were not affected by the hyperthyroid state. However, for propranolol and metoprolol, which undergo extensive presystemic clearance, the AUC values were markedly reduced in the hyperthyroid state.

On basis of the present study, the following conclusions may be drawn:

- There is a defect in suppressor T lymphocyte function in patients with Graves' disease in the hyperthyroid state. This defect may persist in the euthyroid state after treatment.
- The thionamides MMI and PTU inhibit thyroid peroxidase activity, and MMI is a stronger inhibitor than PTU. CMI is essentially inactive *per se* but is bio-activated to MMI. CMI offers no therapeutic advantage over MMI, either from a pharmacodynamic or from a pharmacokinetic point of view. The β -blocker propranolol does not inhibit thyroid peroxidase activity.
- PTU and MMI interfere with mitogenic activation of lymphocytes *in vitro*. This indicates that PTU and MMI may have immunomodulatory effects *in vivo*.
- Propranolol suppresses mitogenic activation of lymphocytes *in vitro*, whereas atenolol has no such effect. This effect of propranolol is related to membrane stabilization rather than to β -adrenoceptor blockade. Thus, β -blockers may have immunomodulatory effects in relation to their membrane stabilizing properties.
- Hyperthyroidism does not affect the kinetics of MMI or atenolol but reduces the AUC values of propranolol and metoprolol. This may be due to enhanced pre-systemic clearance of the latter β -blockers during hyperthyroidism.

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F. REFERENCES

- Adams DD. Bioassay of long-acting thyroid stimulator (L.A.T.S.); the dose-response relationship. *J Clin Endocrinol* 1961;21:799-805.
- Adams DD, Purves HD. Abnormal responses in the assay of thyrotrophin. *Proc Univ Otago Med Sch* 1956;34:11-2.
- Allison AC, Denman AM, Barnes RD. Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet* 1971;II:135-40.
- Almqvist S, Melander A. Läkemedel vid tyreoidesjukdomar. Socialstyrelsens kommitté för läkemedelsinformation 1980;4:1-69.
- Aoki N, Pinnamaneni KM, DeGroot LJ. Studies on suppressor cell function in thyroid diseases. *J Clin Endocrinol Metab* 1979;48:803-10.
- Aro A, Anttila M, Korhonen T, Sundquist H. Pharmacokinetics of propranolol and sotalol in hyperthyroidism. *Eur J Clin Pharmacol* 1982;21:373-7.
- Bach M-A, Bach J-F. The use of monoclonal anti-T cell antibodies to study T cell imbalances in human diseases. *Clin Exp Immunol* 1981;45:449-56.
- Bach M-A, Phan-Dinh-Tuy F, Tournier E, Chatenoud L, Bach J-F. Deficit of suppressor T cells in active multiple sclerosis. *Lancet* 1980;II:1221-3.
- Balázs Cs, Leövey A, Bordan L. Decrease of concanavalin-A activated and short lived suppressor T cell function in thyrotoxicosis. *Biomedicine* 1979;30:143-7.
- Balázs C, Leövey A, Szabó M, Bakó G. Stimulating effect of triiodothyronine on cell-mediated immunity. *Eur J Clin Pharmacol* 1980;17:19-23.
- Balzer J, Lahrtz Hg, van Zwieten PA. Serumspiegel und Urinausscheidung von ¹⁴C-Thiamazol bei Patienten mit Schilddrüsenüberfunktion. *Dtsch Med Wochenschr* 1975;100:548-52.
- Bech K, Nistrup Madsen S. Influence of treatment with radioiodine and propylthiouracil on thyroid stimulating immunoglobulins in Graves' disease. *Clin Endocrinol* 1980;13:417-24.
- Beck JS, Young RJ, Simpson JG, Gray ES, Nicol AG, Pegg CAS, Michie W. Lymphoid tissue in the thyroid gland and thymus of patients with primary thyrotoxicosis. *Br J Surg* 1973;60:769-71.
- Bell JM, Russell CJ, Nelson JK, Kelly JG, McDevitt DG. Studies of the effect of thyroid dysfunction on the elimination of β -adrenoceptor blocking drugs. *Br J Clin Pharmacol* 1977;4:79-82.

- Benner MH, Enta T, Lockey S Jr, Makino S, Reed CE. The immunosuppressive effect of epinephrine and the adjuvant effect of beta-adrenergic blockade. *J Allergy* 1968;41:110-1.
- Bj rum A. Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968;21 suppl 97:9-89.
- Bresnihan B, Jasin HE. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J Clin Invest* 1977;59:106-16.
- Canonica GW, Melioli G, Ferrini S, Trucchi M, Dirienzo W, Bagnasco M. Evaluation of Fc gamma-positive T lymphocytes in Graves' disease: preliminary report. *IRCS Medical Science* 1981;9:172-3.
- Crooks J, Hedley AJ, Macnee C, Stevenson IH. Changes in drug metabolizing ability in thyroid disease. *Br J Pharmacol* 1973;49:156P-7.
- Davidson B, Soodak M, Neary JT, Strout HV, Kieffer JD, Mover H, Maloof F. The irreversible inactivation of thyroid peroxidase by methylmercaptoimidazole, thiouracil, and propylthiouracil *in vitro* and its relationship to *in vivo* findings. *Endocrinology* 1978;103:871-82.
- DeGroot LJ. Short-term antithyroid drug therapy. *N Engl J Med* 1977;297:212-3.
- Denman AM. Lymphocyte function and disease. *Br Med J* 1978;II:980-2.
- Doniach D. Humoral and genetic aspects of thyroid autoimmunity. *Clin Endocrinol Metab* 1975;4:267-85.
- Dutton RW. Inhibitory and stimulatory effects of concanavalin A on the response of mouse spleen cell suspensions to antigen. I. Characterization of the inhibitory cell activity. *J Exp Med* 1972;136:1445-60.
- Editorial. Identifying T cells in man. *Lancet* 1980;II:781-2.
- Eichelbaum M. Drug metabolism in thyroid disease. *Clin Pharmacokinet* 1976;1:339-50.
- Endo K, Kasagi K, Konishi J, Ikekubo K, Okuno T, Takeda Y, Mori T, Torizuka K. Detection and properties of TSH-binding inhibitor immunoglobulins in patients with Graves' disease and Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 1978;46:734-9.
- Ervik M. Quantitative determination of metoprolol in plasma and urine by gas chromatography. *Acta Pharmacol Toxicol* 1975;36 suppl V:136-44.
- Feely J, Crooks J, Stevenson IH. Altered pharmacokinetics of propranolol in hyperthyroidism. *Br J Clin Pharmacol* 1979;8:387P-8.
- Feely J, Crooks J, Stevenson IH. Plasma propranolol steady state concentrations in thyroid disorders. *Eur J Clin Pharmacol* 1981;19:329-33.

- Feighery C, Whelan CA, Weir DG, Greally JF. *In vitro* studies of suppressor cell function in human peripheral blood mononuclear cells. Clin Exp Immunol 1978; 32:459-65.
- Feizi G, Hashizume K, Roubesh CP, DeGroot LJ. Changes in thyroid-stimulating immunoglobulins during antithyroid therapy. J Clin Endocrinol Metab 1979; 48:572-6.
- Finke R, Wenzel B, Goeber R, Schleusener H. Effect of methimazole and propylthiouracil on mitogen-activated lymphocyte populations. Acta Endocrinol 1981; 97 suppl 243:abstract no 330.
- Forfar JC, Pottage A, Toft AD, Irvine WJ, Clements JA, Prescott LF. Paracetamol pharmacokinetics in thyroid disease. Eur J Clin Pharmacol 1980;18:269-73.
- Geffner DL, Azukizawa M, Hershman JM. Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. J Clin Invest 1975;55:224-9.
- Gillette JR. Drug toxicity as a result of interference with physiological control mechanisms. Ann NY Acad Sci 1965;123:42-54.
- Graves RJ. Clinical lectures delivered during the sessions of 1834-5 and 1836-7. Dunglison's American Medical Library. Philadelphia: Adam Waldie, 1838.
- Green WL. Mechanisms of action of antithyroid compounds. In: Werner SC, Ingbar SH, eds. The thyroid. Hagerstown: Harper & Row Publishers, 1978:77-87.
- Grinblat J, Shohat B, Lewitus Z, Joshua H. Quantitative and functional assessment of peripheral T-lymphocytes in thyroid diseases. Acta Endocrinol 1979; 90:52-61.
- Hadden JW, Hadden EM, Middleton E Jr. Lymphocyte blast transformation.
I. Demonstration of adrenergic receptors in human peripheral lymphocytes. Cell Immunol 1970;1:583-95.
- Hayward AR, Layward L, Lydyard PM, Moretta L, Dagg M, Lawton AR. Fc-receptor heterogeneity of human suppressor T cells. J Immunol 1978;121:1-5.
- Heyma P, Larkins RG, Higginbotham L, Wahng K. D-Propranolol and DL-propranolol both decrease conversion of L-thyroxine to L-triiodothyronine. Br Med J 1980; 281:24-5.
- How J, Topliss DJ, Lewis M, Row VV, Volpé R. Graves' disease (GD) may be characterized by an inherited defect in suppressor T lymphocyte function. Ann Endocrinol 1981;42:28A.
- Janosy G, Greaves MF. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clin Exp Immunol 1971;9:483-98.

- Jansson R, Dahlberg P-A, Johansson H, Lindström B. Intratyroidal koncentration av metimazol. *Acta Societatis Medicorum Suecanae Hygiea* 1981a;90:133.
- Jansson R, Dahlberg P-A, Lindström B. Farmakokinetik och peroral tillgänglighet för metimazol och karbimazol. *Acta Societatis Medicorum Suecanae Hygiea* 1981b;90:200.
- Johnsson G, Regårdh CG. Clinical pharmacokinetics of β -adrenoceptor blocking drugs. *Clin Pharmacokinet* 1976;1:233-63.
- Kampmann JP, Mølholm Hansen J. Clinical pharmacokinetics of antithyroid drugs. *Clin Pharmacokinet* 1981;6:401-28.
- Kampmann J, Skovsted L. The kinetics of propylthiouracil in hyperthyroidism. *Acta Pharmacol Toxicol* 1975;37:201-10.
- Keast D, Rennie D, Weetman AP, Foord S, Hall R. The influence of methimazole on autoimmune thyroid disease in the rat. *Ann Endocrinol* 1981;42:66A.
- Kendall-Taylor P. LATS and human-specific thyroid stimulator; their relation to Graves' disease. *Clin Endocrinol Metab* 1975;4:319-39.
- Kidd A, Okita N, Row VV, Volpé R. Immunologic aspects of Graves' and Hashimoto's diseases. *Metabolism* 1980;29:80-99.
- Kriss JP, Pleshakov V, Chien JR. Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. *J Clin Endocrinol* 1964;24:1005-28.
- Kuzuya N, Chiu SC, Ikeda H, Uchimura H, Ito K, Nagataki S. Correlation between thyroid stimulators and 3,5,3'-triiodothyronine suppressibility in patients during treatment for hyperthyroidism with thionamide drugs: comparison of assays by thyroid-stimulating and thyrotropin-displacing activities. *J Clin Endocrinol Metab* 1979;48:706-11.
- Lamberg B-A, Gordin A, Viherkoski M, Kvist G. Long-acting thyroid stimulator (LATS) in toxic nodular goitre, toxic adenoma and Graves' disease. *Acta Endocrinol* 1969;62:199-209.
- Lamberg B-A, Wägar G, Mäkinen T, Gordin A, Apter L. Thyroid stimulating antibodies (TSAb) in Graves' disease and in toxic nodular goitre. *Excerpta Medica. International congress series. No 502. Proc of the XIV internat congress of internal medicine.* 1979:1022-4.
- Lamki L, Row VV, Volpé R. Cell-mediated immunity in Graves' disease and in Hashimoto's thyroiditis as shown by the demonstration of migration inhibition factor (MIF). *J Clin Endocrinol Metab* 1973;36:358-64.

- Landsberg L. Catecholamines and the sympathoadrenal system. In: Werner SC, Ingbar SH, eds. The thyroid. Hagerstown: Harper & Row Publishers, 1978:791-9.
- Lawson A, Rimington C, Searle CE. Antithyroid activity of 2-carbethoxythio-1-methylglyoxaline. Lancet 1951;II:619-21.
- Lazarus JH, Marchant B, Alexander WD, Clark DH. ³⁵S-antithyroid drug concentration and organic binding of iodine in the human thyroid. Clin Endocrinol 1975;4:609-15.
- Lundell G, Wasserman J, Granberg P-O, Blomgren H. Lymphocyte populations in peripheral blood in hyperthyroid and euthyroid subjects. Clin Exp Immunol 1976;23:33-9.
- Lundquist I, Josefsson J-O. Sensitive method for determination of peroxidase activity in tissue by means of coupled oxidation reaction. Anal Biochem 1971;41:567-77.
- Maciel RMB, Miki SS, Nicolau W, Mendes NF. Peripheral blood T and B lymphocytes, *in vitro* stimulation with phytohemagglutinin, and sensitization with 2,4-dinitrochlorobenzene in Graves' disease. J Clin Endocrinol Metab 1976;42:583-7.
- Mäkinen T, Wägar G, Apter L, von Willebrand E, Pekonen F. Evidence that the TSH receptor acts as a mitogenic antigen in Graves' disease. Nature 1978;275:314-5.
- Manley SW, Bourke JR, Hawker RW. The thyrotrophin receptor in guinea-pig thyroid homogenate: interaction with the long-acting thyroid stimulator. J Endocrinol 1974;61:437-45.
- Marchant B, Alexander WD, Lazarus JH, Lees J, Clark DH. The accumulation of ³⁵S-antithyroid drugs by the thyroid gland. J Clin Endocrinol 1972;34:847-51.
- Marchant B, Lees JFH, Alexander WD. Antithyroid drugs. Pharmacol Ther 1978;3:305-48.
- McGavack TH, Chevalley J. Untoward hematologic responses to the antithyroid compounds. Am J Med 1954;17:36-40.
- McGregor AM, Borst GC, Wistar R, Fauci AS. Influence of methimazole on thyroglobulin-induced triggering of lymphocyte function. Ann Endocrinol 1981;42:46A.
- McGregor AM, Ibbertson HK, Smith BR, Hall R. Carbimazole and autoantibody synthesis in Hashimoto's thyroiditis. Br Med J 1980a;281:968-9.
- McGregor AM, Petersen MM, Capiferri R, Evered DC, Smith BR, Hall R. Effects of radioiodine on thyrotrophin binding inhibiting immunoglobulins in Graves' disease. Clin Endocrinol 1979;11:437-44.

- McGregor AM, Petersen MM, McLachlan SM, Rooke P, Smith BR, Hall R. Carbimazole and the autoimmune response in Graves' disease. *N Engl J Med* 1980b;303:302-7.
- McKenzie JM. The thyroid activator of hyperthyroidism. *Trans Assoc Am Physicians* 1959;72:122-30.
- McMurry JF Jr, Gilliland PF, Ratliff CR, Bourland PD. Pharmacodynamics of propylthiouracil in normal and hyperthyroid subjects after a single oral dose. *J Clin Endocrinol Metab* 1975;41:362-4.
- Melander A. Influence of food on the bioavailability of drugs. *Clin Pharmacokinetics* 1978;3:337-51.
- Moretta L, Mingari MC, Webb SR, Pearl ER, Lydyard PM, Grossi CE, Lawton AR, Cooper MD. Imbalances in T cell subpopulations associated with immunodeficiency and autoimmune syndromes. *Eur J Immunol* 1977;7:696-700.
- Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD. Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. *J Exp Med* 1977;146:184-200.
- Mori H, Amino N, Iwatani Y, Kabutomori O, Asari S, Motoi S, Miyai K, Kumahara Y. Increase of peripheral B lymphocytes in Graves' disease. *Clin Exp Immunol* 1980;42:33-40.
- Mukhtar ED, Smith BR, Pyle GA, Hall R, Vice P. Relation of thyroid-stimulating immunoglobulins to thyroid function and effects of surgery, radioiodine, and antithyroid drugs. *Lancet* 1975;I:713-5.
- Mulaisho C, Abdou NI, Utiger RD. Lack of T-cell immune abnormalities in peripheral blood lymphocytes in patients with Graves' disease or hypothyroidism. *J Clin Endocrinol Metab* 1975;41:266-70.
- Nakashima T, Taurog A. Rapid conversion of carbimazole to methimazole in serum; evidence for an enzymatic mechanism. *Clin Endocrinol* 1979;10:637-48.
- Nakazawa H, Adolphson R, Chaperon E, Hobday J, Townley R, Pauli J. In vivo and in vitro effects of chronic propranolol administration on the immune response in mice. *J Allergy Clin Immunol* 1976;57:200.
- Nespoli L, Vitiello A, Maccario R, Lanzavecchia A, Ugazio AG. Activation of human peripheral blood lymphocytes: effect of concanavalin A and lipopolysaccharide on in vitro synthesis of DNA and immunoglobulins. *Scand J Immunol* 1980;12:165-70.
- Nilsson OR, Melander A, Tegler L. Effects and plasma levels of propranolol and metoprolol in hyperthyroid patients. *Eur J Clin Pharmacol* 1980;18:315-20.
- O'Donnell J, Trokoudes K, Silverberg J, Row V, Volpé R. Thyrotropin displacement activity of serum immunoglobulins from patients with Graves' disease. *J Clin Endocrinol Metab* 1978;46:770-7.

- Okita N, Kidd A, Row VV, Volpé R. Sensitization of T-lymphocytes in Graves' and Hashimoto's diseases. *J Clin Endocrinol Metab* 1980;51:316-20.
- Okita N, Row VV, Volpé R. Suppressor T-lymphocyte deficiency in Graves' disease and Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 1981;52:528-33.
- Onaya T, Kotani M, Yamada T, Occhi Y. New *in vitro* tests to detect the thyroid stimulator in sera from hyperthyroid patients by measuring colloid droplet formation and cyclic AMP in human thyroid slices. *J Clin Endocrinol Metab* 1973;36:859-66.
- Parry CH. Diseases of the heart. Enlargement of the thyroid gland in connection with enlargement or palpitation of the heart. In: Collections from the unpublished medical writings. London: Underwoods, 1825;II:111-29.
- Pichler WJ, Broder S. *In vitro* functions of human T cells expressing Fc-IgG or Fc-IgM receptors. *Immunol Rev* 1981;56:163-97.
- Pichler WJ, Lum L, Broder S. Fc-receptors on human T lymphocytes. I. Transition of T_Y to T_μ cells. *J Immunol* 1978;121:1540-8.
- Pinchera A, Liberti P, Martino E, Fenzi GF, Grasso L, Rovis L, Baschieri L, Doria G. Effects of antithyroid therapy on the long-acting thyroid stimulator and the antithyroglobulin antibodies. *J Clin Endocrinol* 1969;29:231-8.
- Polgar PR, Kibrick S, Foster JM. Reversal of PHA-induced blastogenesis in human lymphocyte cultures. *Nature* 1968;218:596-7.
- Pritchard JF, Schneck DW, Hayes AH Jr. Determination of propranolol and six metabolites in human urine by high-pressure liquid chromatography. *J Chromatogr* 1979;162:47-58.
- Pulver W, Bründler R. Erfahrungen mit Methimazole und Carbimazole in der Behandlung von Hyperthyreosen. *Ther Umsch* 1957;14:1-10.
- Reinherz EL, Schlossman SF. Current concepts in immunology. Regulation of the immune response-inducer and suppressor T-lymphocyte subsets in human beings. *N Engl J Med* 1980;303:370-3.
- Reinherz EL, Weiner HL, Hauser SL, Cohen JA, Distaso JA, Schlossman SF. Loss of suppressor T cells in active multiple sclerosis. *N Engl J Med* 1980;303:125-9.
- Rich RR, Pierce CW. Biological expressions of lymphocyte activation. I. Effects of phytomitogens on antibody synthesis *in vitro*. *J Exp Med* 1973;137:205-21.
- Riddell JG, Neill JD, Kelly JG, McDevitt DG. Effects of thyroid dysfunction on propranolol kinetics. *Clin Pharmacol Ther* 1980;28:565-74.
- Roitt IM, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 1956;II:820-1.

- Roitt IM, Ling NR, Doniach D, Couchman KG. The cytoplasmic auto-antigen of the human thyroid. I. Immunological and biochemical characteristics. *Immunology* 1964;7:375-93.
- Rubenfeld S, Silverman VE, Welch KMA, Mallette LE, Kohler PO. Propranolol pharmacokinetics in thyrotoxicosis. *Clin Res* 1978;26:295A.
- Scales B, Copsey PB. The gas chromatographic determination of atenolol in biological samples. *J Pharm Pharmacol* 1975;27:430-3.
- Schuster DL, Pierson D, Bongiovanni B, Levinson AI. Suppressor cell function in atopic dermatitis associated with elevated immunoglobulin E. *J Allergy Clin Immunol* 1979;64:139-45.
- Segal J, Ingbar SH. Stimulation of 2-deoxy-D-glucose uptake in rat thymocytes *in vitro* by physiological concentrations of triiodothyronine, insulin, or epinephrine. *Endocrinology* 1980;107:1354-8.
- Shou L, Schwartz SA, Good RA. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. *J Exp Med* 1976;143:1100-10.
- Siegal FP, Siegal M. Enhancement by irradiated T cells of human plasma cell production: dissection of helper and suppressor functions *in vitro*. *J Immunol* 1977;118:642-7.
- Sitar DS, Abu-Bakare A, Gardiner RJ, Ogilvie RI. Effect of chronic therapy with propylthiouracil on its disposition in hyperthyroid patients. *Clin Invest Med* 1979;2:93-7.
- Sitar DS, Hunninghake DB. Pharmacokinetics of propylthiouracil in man after a single oral dose. *J Clin Endocrinol Metab* 1975;40:26-9.
- Sjöqvist F, Borgå O, Orme ML'E. Fundamentals of clinical pharmacology. In: Avery GS, ed. *Drug treatment*. Edinburgh and London: Churchill Livingstone, 1976:1-42.
- Skellern GG, Knight BI, Low CKL, Alexander WD, McLarty DG, Kalk WJ. The pharmacokinetics of methimazole after oral administration of carbimazole and methimazole, in hyperthyroid patients. *Br J Clin Pharmacol* 1980;9:137-43.
- Skellern GG, Knight BI, Stenlake JB. Improved method for the determination of methimazole in plasma by high-performance liquid chromatography. *J Chromatogr* 1976;124:405-10.
- Skellern GG, Stenlake JB, Williams WD, McLarty DG. Plasma concentrations of methimazole, a metabolite of carbimazole, in hyperthyroid patients. *Br J Clin Pharmacol* 1974;1:265-9.
- Smith BR, Hall R. Thyroid-stimulating immunoglobulins in Graves' disease. *Lancet* 1974;II:427-31.

- Smith CI, Svejgaard A. Concanavalin-A-induced suppressor lymphocytes in normal individuals. *Scand J Immunol* 1981;13:483-92.
- Solomon DH. Antithyroid drugs. In: Werner SC, Ingbar SH, eds. *The thyroid*. Hagerstown: Harper & Row Publishers, 1978:814-21.
- Sridama V, Pacini F, DeGroot LJ. Enumeration of T cells subsets using monoclonal antibodies: decreased percentage of suppressor T cells in Graves' and Hashimoto's disease. *Ann Endocrinol* 1981;42:29A.
- Staehele V, Vallotton MB, Burger A. Detection of human anti-thyroxine and anti-triiodothyronine antibodies in different thyroid conditions. *J Clin Endocrinol Metab* 1975;41:669-75.
- Sugenoya A, Kidd A, Row VV, Volpé R. Correlation between thyrotropin-displacing activity and human thyroid-stimulating activity by immunoglobulins from patients with Graves' disease and other thyroid disorders. *J Clin Endocrinol Metab* 1979;48:398-402.
- Taurog A. The mechanism of action of the thioureyline antithyroid drugs. *Endocrinology* 1976;98:1031-46.
- Teng CS, Yeung RTT. Changes in thyroid-stimulating antibody activity in Graves' disease treated with antithyroid drug and its relationship to relapse: a prospective study. *J Clin Endocrinol Metab* 1980;50:144-7.
- Theilade P, Steiness E, Jørgensen PH, Hansen JM. Decreased first-pass metabolism of propranolol in hyperthyroidism. *World Conf Clin Pharmacol Ther*, London, Abstract no 0608. MacMillan, London, 1980.
- Tötterman TH. Distribution of T-, B-, and thyroglobulin-binding lymphocytes infiltrating the gland in Graves' disease, Hashimoto's thyroiditis, and deQuervain's thyroiditis. *Clin Immunol Immunopathol* 1978;10:270-7.
- Tötterman TH, Mäkinen T, Gordin A. Cell-mediated autoimmunity in thyroid disease as studied by the leukocyte migration test; correlation to clinical state and effect of treatment. *Acta Endocrinol* 1977;86:89-98.
- Urbaniak SJ, Penhale WJ, Irvine WJ. Peripheral blood T and B lymphocytes in patients with thyrotoxicosis and Hashimoto's thyroiditis and in normal subjects. *Clin Exp Immunol* 1974;18:449-59.
- Vanderlaan WP, Storrie VM. A survey of the factors controlling thyroid function, with especial reference to newer views on antithyroid substances. *Pharmacol Rev* 1955;7:301-34.
- Vesell ES, Shapiro JR, Passananti GT, Jorgensen H, Shively CA. Altered plasma half-lives of antipyrine, propylthiouracil, and methimazole in thyroid dysfunction. *Clin Pharmacol Ther* 1975;17:48-56.

- Volpé R. The role of autoimmunity in hypoendocrine and hyperendocrine function. With special emphasis on autoimmune thyroid diseases. *Ann Intern Med* 1977; 87:86-99.
- Volpé R, Row VV. Proportion of E rosettes normal in Graves's and Hashimoto's diseases: a retraction. *N Engl J Med* 1975;293:44.
- von Basedow CA. Exophthalmos durch Hypertrophie des Zellgewebes in der Augenhöhle. *Wschr gesammte Heilkunde* 1840;6:197-204.
- Waal-Manning HJ. Hypertension: Which beta-blocker? *Drugs* 1976;12:412-41.
- Waldmann TA, Blaese RM, Broder S, Krakauer RS. Disorders of suppressor immunoregulatory cells in the pathogenesis of immunodeficiency and autoimmunity. *Ann Intern Med* 1978;88:226-38.
- Wall JR, Manwar GL, Greenwood DM, Walters BA. The in vitro suppression of lectin induced ³H-thymidine incorporation into DNA of peripheral blood lymphocytes after the addition of propylthiouracil. *J Clin Endocrinol Metab* 1976;43:1406-9.
- Wara DW, Reiter EO, Ammann AJ, Kaplan SL. T-cell rosettes in thyrotoxic Graves' disease. *N Engl J Med* 1973;289:1145.
- Warthin AS. The constitutional entity of exophthalmic goiter and so-called toxic adenoma. *Ann Intern Med* 1928;2:553-70.
- Wartofsky L, Dimond RC, Noel GL, Frantz AG, Earll JM. Failure of propranolol to alter thyroid iodine release, thyroxine turnover, or the TSH and PRL responses to thyrotropin-releasing hormone in patients with thyrotoxicosis. *J Clin Endocrinol Metab* 1975;41:485-90.
- Weber TH. Isolation and characterization of a lymphocyte-stimulating leucoagglutinin from red kidney beans. *Scand J Clin Lab Invest* 1969;suppl 111.
- Weiss I, Davies TF. Inhibition of immunoglobulin-secreting cells by antithyroid drugs. *J Clin Endocrinol Metab* 1981;53:1223-8.
- Werner SC. Etiology. In: Werner SC, Ingbar SH, eds. *The thyroid*. Hagerstown: Harper & Row Publishers, 1978a:604-5.
- Werner SC. Other mechanisms. In: Werner SC, Ingbar SH, eds. *The thyroid*. Hagerstown: Harper & Row Publishers, 1978b:624-6.
- Werner SC, Ingbar SH. Hyperthyroidism. In: Werner SC, Ingbar SH, eds. *The Thyroid*. Hagerstown: Harper & Row Publishers, 1978:589.
- Wiberg JJ, Nuttall FQ. Methimazole toxicity from high doses. *Ann Intern Med* 1972;77:414-6.
- Yee Y-G, Rubin P, Blaschke TF. Atenolol determination by high-performance liquid chromatography and fluorescence detection. *J Chromatogr* 1979;171:357-62.

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Suppressor T Lymphocyte Function in Graves' Disease

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ABSTRACT. To explore suppressor T lymphocyte function in Graves' disease, studies were performed in one group of patients in the hyperthyroid state and in another group in the euthyroid state after treatment. Peripheral blood lymphocytes were cultured for 1-7 days. Pokeweed mitogen (PWM; 1.25 μ g/ml) was added at the initiation of the cultures or after 24 h. The degree of lymphocyte activation was assessed by measurements of the cellular uptake of [3 H] thymidine and expressed in counts per minute (cpm). The suppressor lymphocyte function was estimated by a quotient between the maximum cpm values from cultures with and without preincubation.

For the hyperthyroid group (n = 15) the quotient was 1.00 ± 0.07 (mean $\pm$ SEM), for the euthyroid patient group (n = 21) 1.12 ± 0.05 and for the healthy control group (n = 21) 1.37 ± 0.08 . There was a significant difference between the quotients for the control group and the hyperthyroid ($p < 0.01$) as well as the euthyroid ($p < 0.05$) patient group. The quotients for the two groups of patients did not differ significantly.

In conclusion, the present study supports the view of a defect in suppressor T lymphocyte function in patients with Graves' disease in the hyperthyroid state and indicates that this defect can persist in the euthyroid state after treatment.

There is reason to assume that the pathogenesis of Graves' disease has an immunological origin (Kidd et al. 1980) and it has been suggested that it could be attributed to a defect in suppressor T lymphocyte function (Volpé 1977). As suppressor cells are short-lived or become functionally ineffective after 24 h of incubation in culture (Bresnihan & Jasin 1977; Dutton 1972; Feighery et al. 1978) the suppressor T lymphocyte function can be evaluated by comparing mitogenic activation of lymphocytes with and without preincubation (Bresnihan & Jasin 1977). Using this technique Aoki et al. (1979) and Balázs et al. (1979) recently reported that the suppressor cell function was impaired in hyperthyroid patients with Graves' disease.

In the present study the suppressor T lymphocyte function was investigated in one group of patients with Graves' disease in the hyperthyroid state, and in another group in the euthyroid state after treatment. The influence of 3,5,3'-triiodothyronine (T_3) on mitogenic activation of lymphocytes was also assessed.

MATERIAL AND METHODS

Subjects

a) Suppressor study

Fifteen patients (mean age 48 years; range 25-72 years; 5 males and 10 females) with hyperthyroid Graves' disease were studied. The diagnosis of Graves' disease was based on clinical examination, determination of serum concentrations of T_3 and thyroxine (T_4) measured by radioimmunoassay (RIA) (Thorell & Larsson 1978) and thyroid scintigraphy. Eleven of the patients had not been treated for their hyperthyroidism, 2 had relapsed after treatment with thionamides and 2 were previously treated with radioiodine. They were all hyperthyroid at the time of the study and all had a homogeneous distribution of radioactivity on scintigraphy (Table 1).

Table 1. Clinical and biochemical characteristics of the patient groups

	T_3 nmol/liter* (Mean $\pm$ SEM)	T_4 nmol/liter** (Mean $\pm$ SEM)	TSH mIU/liter*** (Mean $\pm$ SEM)	Previous principal therapy with regard to hyper- thyroidism Thion- amides	Radio- iodine Surgery	Observation time in months after principal therapy (Mean $\pm$ SEM) Range
Hyperthyroid						
Graves' disease						
(n = 15)	6.1 $\pm$ 0.6	238 $\pm$ 4	1.6 $\pm$ 0.2	2	- 2	9 $\pm$ 6 1-26
Euthyroid						
Graves' disease						
(n = 21)	1.9 $\pm$ 0.1	133 $\pm$ 8	5.6 $\pm$ 1.6	6	3 12	25 $\pm$ 5 3-94

* Reference range 0.9 - 3.2

** " 50 - 150

*** " < 8

Studies were also performed in 21 patients (mean age 44 years; range 23-67 years; 3 males and 18 females), who had been previously treated for hyperthyroid Graves' disease but at the time of the study were clinically euthyroid and presented normal serum levels of thyroid hormones and thyrotropin (TSH) measured by RIA (Thorell & Larsson 1978) (Table 1). Eight of these patients were on continuous thyroxine therapy.

Twenty-one (mean age 35 years; range 25-60 years; 9 males and 12 females) clinically healthy laboratory subjects served as controls.

b) T_3 study

Blood samples were obtained from clinically healthy laboratory subjects ($n = 10$).

Lymphocyte sampling

Lymphocytes were obtained from heparinized venous blood samples (40-60 ml). The cells were prepared by centrifugation on Ficoll-Isopaque (Lymphoprep, Nyegaard & Co A/S, Oslo, Norway) as described by Bøyum (1968).

Lymphocyte culture conditions

Lymphocyte samples were cultured at 37°C in microtiter plates (Linbro Scientific Inc., Hamden, CT, USA) containing 3×10^5 lymphocytes per culture in 200 μ l serum-free RPMI 1640 (Gibco-Bio-Cult, Glasgow, Scotland) with the addition of gentamicin (12 μ g/ml), glutamine (2 mmol/liter) and 1 or 5 g bovine serum albumin per liter.

a) Suppressor study

The lymphocytes were incubated both in the absence and the presence of pokeweed mitogen (PWM; Sigma Chemical Co., St Louis, MO, USA; 1.25 μ g/ml). The PWM concentration was chosen to give suboptimal lymphocyte stimulation. PWM was added either at initiation of culture (non-preincubated culture) or after 24 hours of culture (preincubated culture). The cells were cultured for 1-7 days

and at each 24 hour period a fraction of the cultures was harvested and the DNA synthesis measured.

b) T_3 study

The lymphocytes were incubated for 72 hours in the presence of PWM (2.5 $\mu\text{g/ml}$), phytohaemagglutinin (PHA; Wellcome Ltd., London, England; 1.25 $\mu\text{g/ml}$) or concanavalin A (Con A; Pharmacia Fine Chemicals, Uppsala, Sweden; 5.0 $\mu\text{g/ml}$). The concentrations were chosen to give optimal lymphocyte stimulation and the mitogens were added to the cultures from the beginning.

T_3 was obtained from British Drug House, Poole, England. It was dissolved and diluted in RPMI 1640 and was added to the cultures simultaneously with the mitogens to yield final concentrations of 0.1, 1, 10, 100 and 1000 nmol/liter.

Measurements of DNA synthesis

[^3H] Thymidine (0.5 μCi ; NET-027Z; SA, 48 Ci/mmol; New England Nuclear Corp., Boston, MA, USA) in RPMI 1640 was added 22-24 hours before harvesting the lymphocytes. The cells were harvested on to glass fibre filters and rinsed in distilled water using a Skatron harvesting machine (Lierbyen, Oslo, Norway). The filters were dried 30 min under a heat lamp and transferred to scintillation vials containing 4 ml Insta-Fluor solution (Packard, Downers Grove, IL, USA). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter and expressed in counts per minute (cpm).

Calculation of results

a) Suppressor study

All cultures were carried out in triplicate. The median cpm value of each triplicate minus the median cpm value for corresponding culture without mitogen was calculated. The value thus obtained from the day of maximum response for each culture was used to estimate the suppressor lymphocyte function by the quotient preincubated culture value/non-preincubated culture value.

b) T_3 study

All cultures were carried out in triplicate except controls which were quintuples. The median value of each triplicate was calculated as the percentage of control, i.e. the value derived from culture with mitogen in the absence of T_3 .

Statistics

The statistical significance of differences was calculated by both Student's t-test and Wilcoxon's test for unpaired (suppressor study) and paired (T_3 study) differences. Correlation coefficient (r) was calculated by conventional method. All values are given as mean $\pm$ SEM and the level of significance is taken as $p < 0.05$.

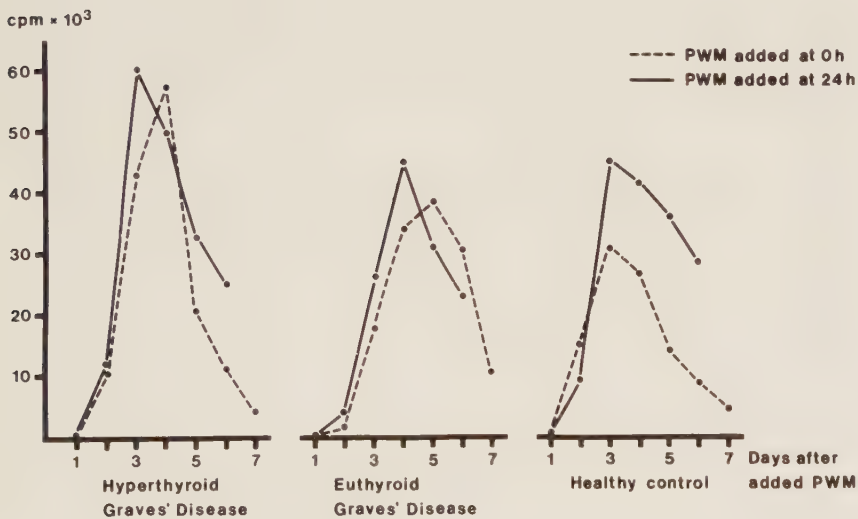


Fig. 1. Typical individual curves of daily uptake of [^3H] thymidine in human peripheral blood lymphocytes activated with PWM 1.25 $\mu\text{g/ml}$ added initially or after 24 hours preincubation.

RESULTS

Figure 1 shows curves of representative single experiments to demonstrate the daily cpm values during 6 (preincubated cultures) or 7 (non-preincubated cultures) days after addition of PWM. The time in days to reach cpm maximum in non-preincubated and preincubated cultures were 3.4 ± 0.16 and 3.5 ± 0.13 in the hyperthyroid group, 4 ± 0.11 and 3.8 ± 0.10 in the euthyroid patient group and 3.7 ± 0.16 and 3.6 ± 0.11 in the control group. The difference between the hyperthyroid and the euthyroid patient group for non-preincubated cultures was significant ($p < 0.02$).

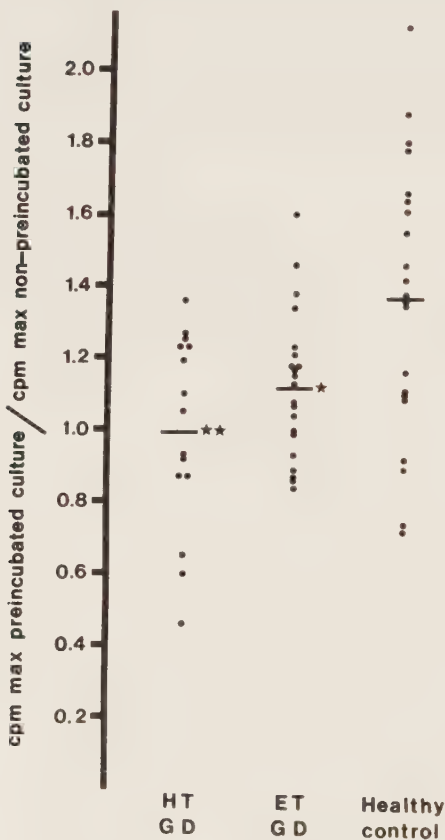


Fig. 2. Individual quotients between maximum cpm values for human peripheral blood lymphocytes activated with PWM $1.25 \mu\text{g/ml}$ with and without 24 hours preincubation. Horizontal lines indicate the mean values. (HT = hyperthyroid, ET = euthyroid, GD = Graves' disease).

* $p < 0.05$ vs control, ** $p < 0.01$ vs control

The quotient for the hyperthyroid group (1.00 ± 0.07) was significantly different ($p < 0.01$) from that of the control group (1.37 ± 0.08) but not from the quotient of the euthyroid patient group (1.12 ± 0.05) (Fig. 2). Also the quotients for the euthyroid patient group and the control group significantly differed ($p < 0.05$) (Fig. 2).

There was no correlation between the recorded quotients and the serum T_3 values for the hyperthyroid group ($r = 0.027$).

T_3 slightly (18%) reduced ($p < 0.05$) PWM-activation ($n = 10$) of peripheral blood lymphocytes at 1000 nmol/liter but had no influence on PHA- ($n = 10$) or Con A- ($n = 7$) activation.

DISCUSSION

Volpé (1977) suggested that Graves' disease and other autoimmune diseases may be due to impaired immunoregulation possibly related to a defect in suppressor T lymphocyte function. To test this hypothesis different methods evaluating the number (Canonica et al. 1981) or the function (Aoki et al. 1979; Balázs et al. 1979; Okita et al. 1981) of suppressor T lymphocytes have been used.

The present finding that 24 hour preincubation of lymphocytes from healthy individuals enhanced the response to mitogen is in accordance with previous studies employing the same technique (Aoki et al. 1979; Balázs et al. 1979; Bresnihan & Jasin 1977; Feighery et al. 1978; Schuster et al. 1979). In addition Aoki et al. (1979) and Balázs et al. (1979) demonstrated that the enhancement after preincubation was significantly decreased using lymphocytes from patients with hyperthyroid Graves' disease compared to lymphocytes from healthy individuals. Our results in the hyperthyroid group agree with those of Aoki et al. (1979) and Balázs et al. (1979) even though these authors in contrast to us used a fixed day after addition of mitogen to compare the cpm values. The mean age of the present hyperthyroid group differed somewhat from that of the control group. However, if the youngest and the oldest persons were excluded and the quotients of the 34-48 years old subjects were compared, there was still a significant difference between the hyperthyroid group ($n = 7$) and the control group ($n = 8$).

The present results in the hyperthyroid group could indicate a defect in suppressor T lymphocytes in Graves' disease. Another possibility is that the recorded effects were due to the hyperthyroid state per se and the study was therefore extended to euthyroid patients previously treated for hyperthyroid Graves' disease. The quotients in this patient group were also significantly different from the control group even if the quotients tended to be somewhat higher than in the hyperthyroid group. This was possibly due to heterogeneity in the group of euthyroid patients (Kidd et al. 1980). Anyhow, the results in the euthyroid patient group indicate that the differences recorded in the hyperthyroid group are not mainly due to the hyperthyroid state per se. This view is further supported by the lack of correlation between the serum T_3 values and the quotients in the hyperthyroid group.

Since Balázs et al. (1980) recently reported an immunostimulatory effect of T_3 we also studied if T_3 could have an immunomodulatory influence. When lymphocytes were activated with different mitogens in the presence of T_3 , we found only a slight suppressive effect on PWM-activation at a supraphysiological concentration of T_3 (1000 nmol/liter), otherwise no effect. Thus, our results based on in vitro studies do not indicate that T_3 has immunomodulatory effects. The reason for the discrepancy between our results and those of Balázs et al. (1980) is not evident.

The present results in the hyperthyroid group and those of Aoki et al. (1979) and Balázs et al. (1979) using the same technique indicate a defect in suppressor T lymphocyte function in hyperthyroid patients with Graves' disease. This view has gained further support by studies of Okita et al. (1981) using a modified migration inhibition factor test for evaluating the suppressor lymphocyte function and of Canonica et al. (1981) counting the number of T cells bearing receptors for the Fc portion of IgG in patients with Graves' disease.

The present results in the euthyroid patient group indicate that the defect in suppressor T lymphocyte function recorded in hyperthyroid Graves' disease can persist after therapy in certain patients.

In conclusion, the present study supports the view of a defect in suppressor T lymphocyte function in patients with Graves' disease in the hyperthyroid state and indicates that this defect can persist in the euthyroid state after treatment.

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REFERENCES

- Aoki N, Pinnamaneni K M & DeGroot L J (1979): Studies on suppressor cell function in thyroid diseases. *J Clin Endocrinol Metab* 48:803-810.
- Balázs Cs, Leövey A & Bordan L (1979): Decrease of concanavalin-A activated and short lived suppressor T cell function in thyrotoxicosis. *Biomedicine* 30:143-147.
- Balázs C, Leövey A, Szabó M & Bakó G (1980): Stimulating effect of triiodo-thyronine on cell-mediated immunity. *Eur J Clin Pharmacol* 17:19-23.
- Bresnihan B & Jasin H E (1977): Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J Clin Invest* 59:106-115.
- Bøyum A (1968): Isolation of leukocytes from human blood. *Scand J Clin Lab Invest* 21 (Suppl 97):9-89.
- Canonica G W, Melioli G, Ferrini S, Trucchi M, Dirienzo W & Bagnasco M (1981): Evaluation of Fc gamma-positive lymphocytes in Graves' disease: Preliminary report. *IRCS Medical Science* 9:172-173.
- Dutton R W (1972): Inhibitory and stimulatory effects of concanavalin A on the response of mouse spleen cell suspensions to antigen. *J Exp Med* 137:1445-1460.
- Feighery C, Whelan C A, Weir D G & Greally J F (1978): In vitro studies of suppressor cell function in human peripheral blood mononuclear cells. *Clin Exp Immunol* 32:459-465.
- Kidd A, Okita N, Row V V & Volpé R (1980): Immunologic aspects of Graves' and Hashimoto's diseases. *Metabolism* 29:80-99.
- Okita N, Row V V & Volpé R (1981): Suppressor T-lymphocyte deficiency in Graves' disease and Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 52:528-533.
- Schuster D L, Pierson D, Bongiovanni B & Levinson A I (1979): Suppressor cell function in atopic dermatitis associated with elevated immunoglobulin E. *J Allergy Clin Immunol* 64:139-145.
- Thorell J I & Larsson S M (1978): Pituitary thyroid axis. In: *Radioimmunoassay and related techniques*, p 109-121. The C.V. Mosby Company, St. Louis, MO.
- Volpé R (1977): The role of autoimmunity in hypopendocrine and hyperendocrine function. *Ann Intern Med* 87:86-99.

Comparative In Vitro Effects and In Vivo Kinetics of Antithyroid Drugs

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Summary. The in vitro effects of equimolar concentrations (0.1, 0.33 and 1.0 mmol/l) of carbimazole, methimazole, propylthiouracil and propranolol on thyroid peroxidase activity were studied on thyroid tissue specimens obtained from euthyroid patients undergoing parathyroidectomy. In addition, the in vivo kinetics of methimazole following single dose administration (60 mg) of carbimazole and of methimazole itself were examined in 11 healthy volunteers using high-pressure liquid chromatography to measure serum methimazole.

The in vitro studies were carried out at pH 6, to avoid alkaline hydrolysis of carbimazole to methimazole. Under these conditions, methimazole strongly inhibited thyroid peroxidase. Propylthiouracil had a less pronounced inhibitory effect, and carbimazole was almost and propranolol was entirely inactive. The in vivo kinetics of methimazole showed a large interindividual variation. Within individuals, there was no significant difference in the half-life or time to peak concentration of methimazole following administration of carbimazole and methimazole, respectively. However, the peak concentration and area under the curve of methimazole were significantly greater after administration of methimazole itself than after administration of carbimazole.

Assuming similar bioavailability, this difference could be related to the difference in molecular weight between carbimazole and methimazole. It appears that, in man, methimazole is the most active of antithyroid agents currently available, that carbimazole is essentially inactive per se but is bioactivated to methimazole, and that carbimazole offers neither dynamic nor kinetic advantages over methimazole.

Key words: propylthiouracil, propranolol, carbimazole, methimazole; comparative activity, pharmacokinetics, bioactivation, thyroid peroxidase inhibition

Thionamide drugs are important tools in the therapy of hyperthyroidism. Currently, the most commonly employed thionamides are carbimazole, methimazole and propylthiouracil. The assumed basis for their antithyroid action is the same, namely they interfere with peroxidase-catalyzed oxidation of iodide, leading to reduced intrathyroidal formation of iodo-tyrosines and iodothyronines [1].

The effect of carbimazole is at least partly mediated by conversion to methimazole [2]. Indeed, following in vivo administration, carbimazole can be detected neither in the thyroid nor in blood, and instead methimazole appears in both compartments [3, 4]. Nevertheless, carbimazole is preferred to methimazole in many European countries. One reason for this appears to be the belief that carbimazole is less toxic and has a more prolonged action than methimazole. Neither of these ideas has ever been documented, and so the following questions emerge: does carbimazole possess any intrinsic antithyroid activity, or does it function only as a pro-drug, being bioactivated to methimazole? If the latter were true, is the kinetics of methimazole following carbimazole administration different from that after intake of methimazole itself? The present study attempts to answer these questions. The antithyroid activity of propylthiouracil and propranolol was also assessed. The latter was included as it is being increasingly used in the treatment of hyperthyroidism.

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Material and Methods

Pharmacodynamic Experiments

Human Thyroid Specimens. Normal thyroid tissue was obtained from three patients undergoing neck surgery for hyperparathyroidism. Consent had been given by the patients. They were euthyroid according to clinical examination and measurement of S-T₃ and S-T₄, and they had received no antithyroid drugs. The specimens were homogenized in cold 0.9% saline, 1 ml per 10 mg wet weight of thyroid tissue, by use of an Ultra-Turrax homogenizer for 30 s. The homogenate was centrifuged for 5 min at 3000 rpm, in a Sorvall centrifuge, at 4 °C. The supernatants were collected, pooled and stored at -20 °C until used for peroxidase assays.

Assay of Human Thyroid Peroxidase Activity. Peroxidase activity in human thyroid tissue was assayed by the method of Lundquist and Josefsson [5], employing a reagent solution containing o-dianisidine, glucose oxidase, Triton-X-100, citric acid and sodium hydroxide, which was added to a mixture of glucose, ammonium hydroxide and calcium chloride when the incubation was initiated. The drugs were dissolved in saline and were added to the medium together with the glucose-ammonia-calcium mixture. Particular care was taken to maintain the pH at 6.0, in order to avoid any un-intentional conversion of carbimazole to methimazole, as may readily occur in plasma and other alkaline media [2, 6]. Previous experiments had shown that in the system used the pH optimum for peroxidase activity in human thyroid was between 6.0 and 6.5. Incubation was carried out at 37 °C for 30 min. The significance of differences was calculated by Student's *t*-test.

Drugs. Carbimazole was obtained from Nicholas Laboratories Ltd., Slough, England; methimazole from AB Kabi, Stockholm, Sweden; 6-propyl-thiouracil from AB Pharmacia, Uppsala, Sweden; and *D*, *L*-propranolol from ICI, Ltd., Macclesfield, England.

Pharmacokinetic Experiments

Subjects. Five female and three male volunteers, 22–37 years old, weight range 52–67 kg, who were clinically healthy, participated in the study. Each subject was extensively informed and gave his written consent to it. The investigation was approved by the Ethical Committee of the University of Lund.

The subjects abstained from any alcoholic beverage for at least two days before and on the day of the experiment. Smokers (2 subjects) were not asked to

refrain from smoking other than at the time of drug ingestion and blood sampling.

Drug Intake. Each subject came to the laboratory twice, at an interval of at least 7 days. On both occasions they arrived at about 8 a. m., having fasted for at least 10 h. On one occasion, carbimazole 60 mg (12 × 5 mg Neo-Mercazole®, Nicholas Laboratories Ltd., Slough, England) was ingested, and on the other methimazole 60 mg (12 × 5 mg Thacapzol®, AB Kabi, Stockholm, Sweden). On both occasions, the tablets were taken immediately after eating a standardized breakfast meal, with a total energy content of 1840 kJ (440 kcal). The order of drug intake was randomized. The nurse who collected the blood samples also supervised the eating and intake of the tablets. No subject reported any adverse effect.

Blood Samples. Blood samples (10 ml) were obtained through an indwelling polythene catheter (see above) before administration of the meal and drug (0 value, see above), and 1/4, 1/2, 1, 1 1/2, 2, 3, 4, 6, 8, 12 and 24 h after drug intake. Blood was collected into Venoject® tubes without any additive and serum prepared by centrifugation for 10 min was kept at 4 °C until assayed for methimazole. The assay was always carried out within 24 h of collecting the blood. Examination of serum samples to which methimazole had been added in vitro showed that methimazole was stable in serum (>95% retained) for 24 h at 4 °C, but that significant degradation (≥25% disappearance) occurred within a week.

Assay of Methimazole. Methimazole in serum was determined by high-pressure liquid chromatography, as described by Skellern et al. [2, 6] with some minor modifications. In brief, benzamide 10 µg in ethyl alcohol 100 µl was added as internal standard to serum 1 ml and redistilled water 1 ml. The sample was slightly shaken. Chloroform 5 ml containing 0.25% *n*-octanol was then added, and the tube was shaken for 15 min and then centrifuged for 10 min at room temperature. The supernatant was pipetted off, and the chloroform layer was filtered into another tube through a Whatman filter 1 p. S. Four ml filtrate were desiccated at 30 °C under a stream of nitrogen in a water bath. The remainder was dissolved in ethyl alcohol 200 µl and shaken. 15–25 µl were injected into the liquid chromatograph, Waters model 6000 A, equipped with a µBondapak C18 column, 10 µm particles, mobile phase 6% ethanol in water, flow rate 1 ml/min, 1500 psi. A Waters model 440 UV-detector was used at a wave-length of 254 nm.

Calculations. Serum concentrations of methimazole were plotted against time. The peak concentration

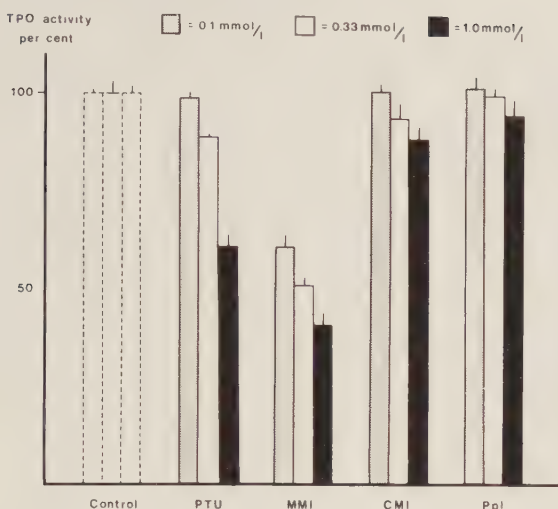


Fig. 1. Influence of equimolar concentrations of 6-propylthiouracil (PTU), methimazole (MMI), carbimazole (CMI) and *D*, *L*-propranolol (Ppl) on thyroid peroxidase (TPO) activity in normal human thyroid tissue. The results are the means of three experiments of similar design with three different doses. Each bar represents mean ($\pm$ SEM) of nine (3×3) observations

(C_{max}) and time to peak concentration (t_{max}) are given as those recorded. The elimination half-life ($t_{1/2}$) was calculated by regression analysis and the area under the serum concentration curve (AUC) from 0 to ∞ was estimated by the trapezoidal rule. The statistical significance of differences was assessed by Student's *t*-test for paired observations.

Results

Pharmacodynamic Experiments

The effect of three different concentrations of propylthiouracil, methimazole, carbimazole and propranolol on human thyroid peroxidase activity is shown in Figure 1. It can be seen that on equimolar basis methimazole exerted a strong inhibitory influence on peroxidase activity, giving a significant ($p < 0.001$) reduction at each dose level. Propylthiouracil had a weaker effect, which was significant at the highest ($p < 0.01$) and the intermediate ($p < 0.05$) but not at the lowest dose. Carbimazole had a slight but significant ($p < 0.05$) inhibition at the highest dose level. Propranolol did not cause significant inhibition at any dose level.

Pharmacokinetic Experiments

The serum concentrations of methimazole in each subject, after administration of methimazole itself

and after an identical dose of carbimazole are shown in Figure 2. The resulting kinetic data are given in Table 1. There were very great interindividual differences in peak concentrations, elimination half-lives and AUCs. Within individuals, there was no significant difference in half-lives or times to peak concentration. However, both the peak concentrations and the AUCs were significantly greater ($p < 0.01$ in paired *t*-tests) following administration of methimazole itself than after intake of carbimazole.

Discussion

Although their precise mechanism of action has not been elucidated, there is evidence that thionamides interfere with thyroid peroxidase activity [1]. In addition, methimazole is a strong irreversible peroxidase inhibitor in man, whereas propylthiouracil works reversibly and is a weaker inhibitor [7]. The present findings support the view that methimazole is a stronger and propylthiouracil a weaker antithyroid agent. Propranolol had no inhibitory influence on thyroid peroxidase activity, which is in agreement with the finding that propranolol does not affect thyroxine biosynthesis [8].

Carbimazole exerted a slight but significant inhibition of thyroid peroxidase activity only at the highest concentration, which was far above any blood level that could conceivably be attained *in vivo* following conventional doses of carbimazole. Animal studies of

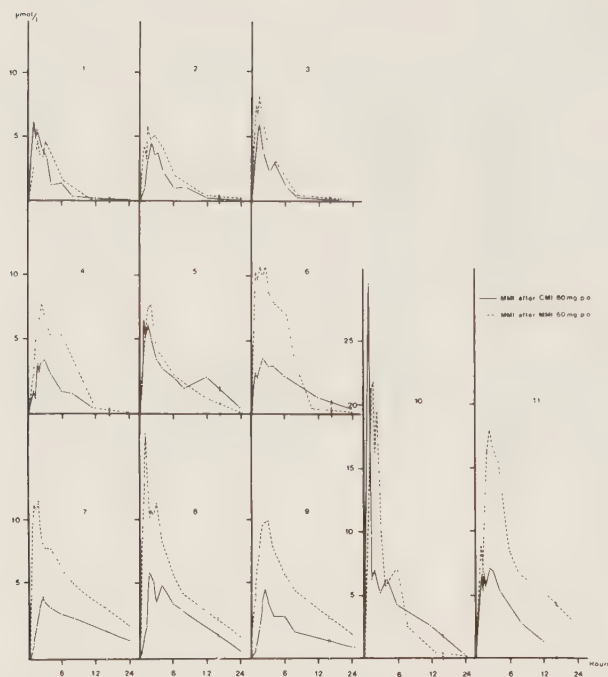


Fig. 2. Serum concentrations of methimazole in eleven healthy subjects after ingestion of carbimazole 60 mg (CMI) and methimazole (MMI) 60 mg. The calculated kinetic data are given in Table 1

Table 1. Kinetic parameters of methimazole in eleven healthy volunteers after ingestion of carbimazole 60 mg (C) and of methimazole 60 mg (M). C_{max} = peak concentration, t_{max} = peak concentration, $t_{1/2}$ = elimination half-life, AUC = area under the serum concentration curve

Subject no.	C_{max} ($\mu\text{mol/l}$)			t_{max} (min)		$t_{1/2}$ (min)		AUC ($0 \rightarrow \infty$) ($\mu\text{mol} \times \text{min} \times \text{l}^{-1}$)		
	C	M	M/C ratio	C	M	C	M	C	M	M/C ratio
1	6.39	4.64	0.73	60	179	135	139	20.86	22.05	1.06
2	4.64	5.87	1.27	123	79	168	180	21.80	33.78	1.55
3	6.04	8.23	1.36	75	75	136	120	22.23	30.26	1.36
4	4.20	8.58	2.04	180	149	174	154	25.25	55.68	2.21
5	7.53	8.50	1.13	45	117	300	261	45.17	51.59	1.14
6	4.38	11.65	2.66	120	149	390	207	47.59	78.20	1.64
7	4.91	12.44	2.53	153	106	570	414	70.67	115.56	1.64
8	6.75	17.70	2.62	105	59	384	240	75.20	99.74	1.33
9	5.43	10.86	2.00	150	179	270	312	36.87	99.02	2.69
10	29.43	21.55	0.73	59	108	291	126	78.95	75.19	0.95
11	7.01	17.87	2.55	149	148	228	312	52.95	149.58	2.82
	$p < 0.01^a$			N.S. ^a		N.S. ^a		$p < 0.01^a$		
	mean: 1.79							mean: 1.67		

^a Level of statistical significance in Student's paired *t*-test on intraindividual data pairs.

peroxidase-mediated iodination in vitro have suggested that carbimazole may have intrinsic antithyroid activity [1]. However, the authors cited do mention the possibility that carbimazole is rapidly hydrolyzed to methimazole [1]. This is very likely to have occurred as the incubation was carried out at pH 7 [1]; hydrolysis of carbimazole to methimazole occurs rapidly at this and higher pH [2, 6]. In fact, following administration in vivo carbimazole has never been detected in blood or in thyroid tissue, but instead, methimazole is found in both compartments [3, 4]. Although the incubations in the present study were carried out at pH 6, to avoid hydrolysis of carbimazole to methimazole, such conversion might still have occurred to some degree. Accordingly, it is reasonable to assume that carbimazole functions as a pro-drug: as such it is inactive and its clinical antithyroid action is exerted only after it has undergone bioactivation to methimazole.

From this it follows that carbimazole offers no pharmacodynamic advantage over methimazole. However, due to slow hydrolysis of carbimazole to methimazole, a sustained release of methimazole might occur in the body, which would favour a longer duration of effect [9]. In addition, it has been considered that the allegedly prolonged effect of carbimazole administration might yield a more constant antithyroid effect with lesser exposure to methimazole [9]. This should help to reduce the risk of side-effects.

The present findings give no support to the sustained release concept. Thus, neither the time to reach the peak concentration nor the elimination half-life of methimazole showed any significant intraindividual difference after administration of carbimazole and methimazole. Therefore, it is unlikely that carbimazole therapy would give more sustained levels of methimazole in blood and tissues than would direct administration of methimazole itself. The only intraindividual differences observed were that both the peak concentrations and the AUCs of methimazole were significantly lower following carbimazole administration than after intake of methimazole. As it is common clinical practice to administer the same quantities of carbimazole and methimazole, the findings indicate that conventional therapy with carbimazole would give less methimazole in the body than would conventional treatment with methimazole. This is not surprising, as the molecular weight of carbimazole (186) is considerably greater than that of methimazole (114). Assuming equal bioavailability of methimazole from the two preparations, methimazole 60 mg should yield 63% more methimazole than should carbimazole 60 mg which would be equivalent only to methimazole 37 mg. The latter figure agrees well with the recorded difference in mean AUC of 67%.

The fact that carbimazole delivers less methimazole to the general circulation than does methimazole may provide a factual basis for the widespread clinical opinion that carbimazole is less toxic than methimazole. However, this merely reflects a reduction in the resulting dose of the active agent and does not signify a true therapeutic advantage. In conclusion, it appears that methimazole is the most active of currently available antithyroid agents in man, that carbimazole is inactive per se but is bioactivated to methimazole, and that carbimazole offers neither dynamic nor kinetic advantages over methimazole.

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References

1. Nakashima T, Taurog A, Riesco G (1978) Mechanism of action of thiourylene antithyroid drugs. *Endocrinology* 103: 2187-2197
2. Skellern GG, Stenlake JB, Williams WD, McLarty DG (1974) Plasma concentrations of methimazole, a metabolite of carbimazole, in hyperthyroid patients. *Br J Clin Pharmacol* 1: 265-269
3. Marchant B, Alexander WD, Lazarus JH, Lees J, Clark DM (1972) The accumulation of ^{35}S -antithyroid drugs by the thyroid gland. *J Clin Endocrinol Metab* 34: 847-852
4. Lazarus JH, Marchant B, Alexander WD, Clark DH (1975) ^{35}S -antithyroid drug concentration and organic binding of iodine in the human thyroid. *Clin Endocrinol* 4: 609-613
5. Lundquist I, Josefsson J-O (1971) Sensitive method for determination of peroxidase activity in tissue by means of coupled oxidation reaction. *Anal Biochem* 41: 567-577
6. Skellern GG, Knight BI, Stenlake JB (1976) Improved method for the determination of methimazole in plasma by high-performance liquid chromatography. *J. Chromatogr* 124: 405-410
7. Nagasaka A, Hidaka H (1976) Effect of antithyroid agents 6-propyl-2-thiouracil and 1-methyl-2-mercaptoimidazole on human thyroid iodide peroxidase. *J Clin Endocrinol Metab* 43: 152-158
8. Lowe DC, Hadden DR, Montgomery DAD, Weaver JA (1976) Propranolol as the sole therapy for thyrotoxicosis: Long term follow up. In: Robbins J, Braverman LE (eds) *Thyroid research*. Excerpta Medica, Amsterdam, pp 429-433
9. Pulver W, Bründler R (1957) Erfahrungen mit Methimazole und Carbimazole in der Behandlung von Hyperthyreosen. *Therap Umschau* 14: 1-10

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Effects of Antithyroid Drugs on Lymphocyte Function *in Vitro**

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ABSTRACT. The *in vitro* influence of 6-propylthiouracil (PTU) and methimazole (MMI) on mitogenic activation of human peripheral blood lymphocytes was studied in order to assess whether antithyroid drugs may have an immunomodulatory capacity.

Lymphocytes were cultured for 72 h in the presence of phytohaemagglutinin (PHA; 1.25 µg/ml), concanavalin A (Con A; 5.0 µg/ml), or pokeweed mitogen (PWM; 2.5 µg/ml). Antithyroid drugs were added to yield final concentrations of 1–1000 µmol/liter. High pressure liquid chromatographic analyses verified that the expected drug concentrations in the incubation media were reached and maintained throughout the incubation period.

The degree of lymphocyte activation was assessed by measurements of the cellular uptake of [³H]thymidine added after 48 h.

PTU (at 1000 µmol/liter) slightly suppressed PHA- and Con A-induced activation of lymphocytes but enhanced (at 5–500 µmol/liter) PWM-induced activation. MMI (500–1000 µmol/liter) enhanced not only PWM induced activation but (at 1000 µmol/liter) also enhanced PHA- and Con A-induced activation.

Thus, PTU and MMI might interfere with mitogenic activation of lymphocytes. However, the effects were mainly recorded at drug concentrations exceeding those found in blood samples of patients treated with conventional doses. (*J Clin Endocrinol Metab* 51: 298, 1980)

THE PATHOGENESIS of Graves' disease is still unknown, but there is increasing evidence that it is associated with immunological disturbances (1, 2). Antithyroid drugs are used in the treatment of this disease. The commonly accepted basis for this use is that they inhibit thyroid hormone biosynthesis (3). Hence, they alleviate the hyperthyroid symptoms, while the immunological disturbance is believed to subside spontaneously (4). However, it has been suggested that antithyroid drugs also have a direct immunomodulatory influence and, hence, have an impact on the development of the disease (2, 5–7). It was recently reported that the antithyroid agent propylthiouracil [propyl-2-thiouracil (PTU)] could exert an immunosuppressive effect, as judged by its pronounced influence on *in vitro* mitogenic activation of peripheral blood lymphocytes from healthy subjects (8). The present study compared the influence of PTU and another antithyroid agent, methimazole (MMI), on mitogenic activation of peripheral blood lymphocytes from healthy subjects. The influence of PTU on lymphocytes was also studied in hyperthyroid patients.

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Materials and Methods

Lymphocyte sampling

Lymphocytes from clinically euthyroid individuals were obtained from the buffy coat of fresh donor blood ($n = 6$; using acid citrate dextrose (Pharmacia, Uppsala, Sweden) as anticoagulant) or from heparinized venous blood samples (40 ml) from laboratory personnel ($n = 13$).

In addition, heparinized venous blood samples (40 ml) were obtained from hyperthyroid patients. They were all female, with an age range of 37–80 yr. All patients had clinical symptoms, and their serum concentrations of T_4 were well within the hyperthyroid range. One of them had toxic multinodular goiter, one had thyroiditis (subacute type), and the other three had Graves' disease. They were drug free, except for one patient taking digoxin and furosemide.

Lymphocytes were prepared by centrifugation on Ficoll-Isoopaque (Lymphoprep, Nyegaard and Co. A/S, Oslo, Norway), as described by Bøyum (9).

Lymphocyte cultures

Lymphocyte samples were cultured in microtiter plates (Linbro Scientific, Inc., Hamden, CT) containing 2.5×10^5 lymphocytes/culture in 200 µl serum-free RPMI 1640 (Gibco-Bio-Cult, Glasgow, Scotland) with the addition of gentamicin (12 µg/ml), glutamine (2 mmol/liter), and 10% heat-inactivated pooled normal human serum.

The lymphocytes were incubated in the presence of phytohaemagglutinin (PHA; Wellcome Ltd., London, England; 1.25 µg/ml), concanavalin A (Con A; Pharmacia Fine Chemicals,

Uppsala, Sweden; 5.0 $\mu\text{g/ml}$), or pokeweed mitogen (PWM; Sigma chemical Co., St. Louis, MO; 2.5 $\mu\text{g/ml}$). The concentrations were chosen to give optimal lymphocyte stimulation.

Measurements of DNA synthesis

[^3H]Thymidine (0.5 μCi ; NET-027Z; SA, 48 Ci/mmol; New England Nuclear Corp., Boston, MA) in RPMI 1640 was added after 48 h of culture, i.e. 22–24 h before termination of cultures. The cells were harvested on to glass fiber filters and rinsed in distilled water using a Skatron harvesting machine (Lierbyen Oslo, Norway). The filters were dried 30 min under a heat lamp and transferred to scintillation vials containing 4 ml Insta-Fluor solution (Packard, Downers Grove, IL). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter.

All cultures were carried out in triplicate. The median value of each triplicate was calculated as the percentage of control, i.e. the value derived from culture with lectin in the absence of antithyroid drugs, and are shown together with the SEM.

Drugs

PTU (Tiotil; batch R 720352, Pharmacia) and MMI (Thacapzol, batch A 32639-01, AB Kabi, Stockholm, Sweden) were used. The drugs, both of 99.9% purity, were dissolved by adding them to RPMI 1640 and slowly rotating the mixture at 37°C for 1–2 h. Appropriate dilutions were then made with RPMI 1640. Drug solutions were added to cultures initially and, after 47.5 h, to identical parallel cultures. The various concentrations are given in Results.

Measurements of drug concentrations

To examine whether the drug concentrations were maintained during the whole incubation period, the concentrations of MMI and PTU in the media were measured at regular intervals. The MMI concentrations were determined by high pressure liquid chromatography, as described by Skellern *et al.* (10, 11) with minor modifications (12). The PTU concentrations were determined by a modification of the MMI method. In brief, extraction was carried out with chloroform containing 0.25% *n*-octanol. The dried residue was dissolved in 100 μl ethyl alcohol, and 10 μl were injected into the liquid chromatograph (Varian 8500, Varian Instruments, Palo Alto, CA). The column used was an HCH-10. A Waters model 400 UV-detector was used (Waters Associates, Milford, MA); detection wavelength was 280 nm.

Statistics

The statistical significance of differences was calculated by Student's *t* test.

Results

Influence of PTU and MMI on mitogen-activated lymphocytes

PHA-activated lymphocytes. Figure 1 shows the influence of PTU (5, 50, 500, and 1000 $\mu\text{mol/liter}$) and MMI

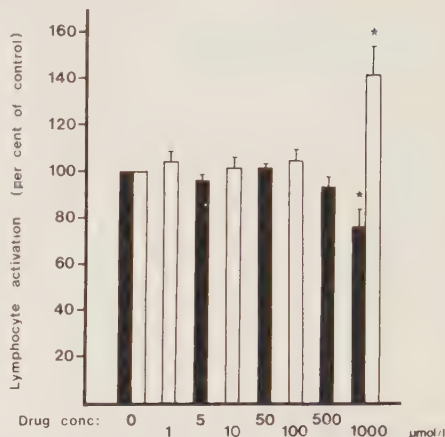


FIG. 1. Uptake of [^3H]thymidine (range, 21,678–175,456 cpm) in normal human peripheral blood lymphocytes activated with PHA (1.25 $\mu\text{g/ml}$) in the presence and absence of PTU (■) or MMI (□; mean $\pm$ SEM; $n = 7$). *, $P < 0.05$.

(1, 10, 100, and 1000 $\mu\text{mol/liter}$) on PHA activation of peripheral blood lymphocytes from healthy subjects after concomitant addition of drug and mitogen. At 1000 $\mu\text{mol/liter}$, but not at the lower concentrations, PTU slightly reduced ($P < 0.02$) whereas MMI augmented ($P < 0.02$) PHA-induced accumulation of [^3H]thymidine. PTU had similar effects when using peripheral blood lymphocytes from hyperthyroid patients; at 1000 $\mu\text{mol/liter}$ PTU evoked a 23% reduction ($P < 0.001$). MMI was not tested against peripheral blood lymphocytes from hyperthyroid subjects.

Con A-activated lymphocytes. Figure 2 demonstrates the influence of PTU and MMI (5, 50, 500, and 1000 $\mu\text{mol/liter}$) on Con A activation of peripheral blood lymphocytes from healthy individuals after concurrent administration of drug and mitogen. At 1000 $\mu\text{mol/liter}$, PTU reduced ($P < 0.02$) whereas MMI augmented ($P < 0.05$) Con A-induced accumulation of [^3H]thymidine.

PWM-activated lymphocytes. Both PTU and MMI (tested at 5, 50, 500, and 1000 $\mu\text{mol/liter}$) appeared to have a stimulatory effect on PWM activation of peripheral blood lymphocytes from healthy subjects (Fig. 3). PTU significantly enhanced [^3H]thymidine accumulation at 5 ($P < 0.02$), 50 ($P < 0.005$), and 500 $\mu\text{mol/liter}$ ($P < 0.02$) and MMI at 500 ($P < 0.01$) and 1000 $\mu\text{mol/liter}$ ($P < 0.005$). Drugs and mitogen were added concurrently.

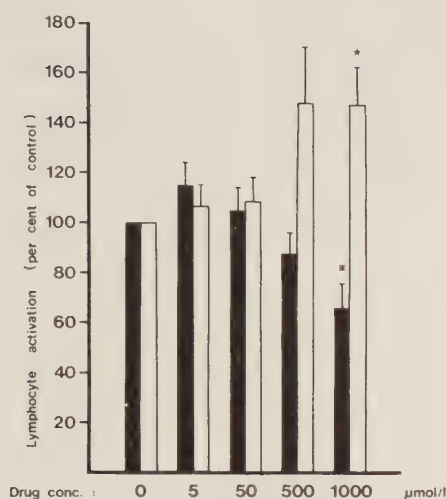


Fig. 2. Uptake of [3 H]thymidine (range, 14,702–114,948 cpm) in normal human peripheral blood lymphocytes activated with Con A (5.0 µg/ml) in the presence and absence of PTU (■; $n = 6$) or MMI (□; $n = 5$; mean $\pm$ SEM). *, $P < 0.05$.

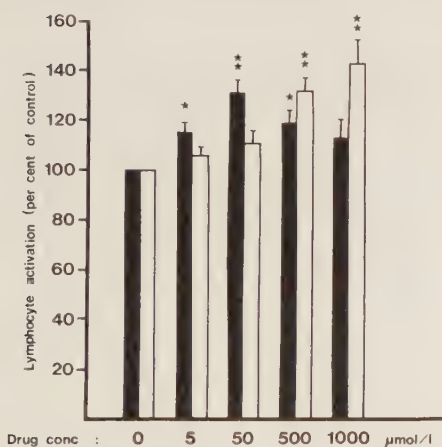


Fig. 3. Uptake of [3 H]thymidine (range, 32,010–122,461 cpm) in normal human peripheral blood lymphocytes activated with PWM (2.5 µg/ml) in the presence and absence of PTU (■; $n = 6$) or MMI (□; $n = 5$; mean $\pm$ SEM). *, $P < 0.05$; **, $P < 0.01$.

Drugs added after 47.5 h. When PTU or MMI was added after 47.5 h, the effects on mitogenic activation of peripheral blood lymphocytes were similar to those recorded when the drugs had been added initially.

Drug concentrations

Measurements of the concentrations of PTU and MMI in the media at regular intervals throughout an experiment revealed that the desired concentrations had been reached and were maintained throughout the incubation time (Table 1).

Discussion

Wall *et al.* (8) recently reported that PTU can suppress PHA-, Con A-, and PWM-induced activation of peripheral blood lymphocytes from healthy subjects using an experimental model similar to that used in the present study. As far as PTU is concerned, the present data do not support those of Wall *et al.* (8). Thus, although PTU was found to suppress PHA- and Con A-induced activation of peripheral blood lymphocytes from healthy subjects, a significant suppressive effect was recorded only at the highest PTU concentration (1000 µmol/liter), whereas Wall *et al.* (8) reported a suppressive effect at 5.88 µmol/liter (1 mg/liter). Another discrepancy was that Wall *et al.* (8) observed maximum suppressive effects when PTU was added after 47.5 h of incubation, while the present findings indicated similar effects after PTU addition at 47.5 h and after concurrent administration of drug and mitogen.

Another difference was that PTU strongly suppressed PWM-induced activation of lymphocytes in the study of Wall *et al.* (8) but slightly enhanced PWM activation in the present investigation. There is no obvious explanation of these differences concerning the effect of PTU. One possibility would be that the drug was degraded in

TABLE 1. Concentrations of PTU and MMI in the culture media after different hours of incubation

Desired conc. (µmol/liter)	Culture time (h)				
	0	5	24	48	72
PTU					
0	0				0
5	5	3	6	5	7
50	49	44	46	51	67
500	488	438	394	482	610
1000		811	864	934	1075
MMI					
0	2				1
1	1	1	1	1	1
10	10	10	10	9	10
100	96	96	103	91	112
1000	915	885	933	955	1012

the medium used in the present study. However, this could be excluded, since measurements of the drug concentrations showed that the desired concentrations were reached and maintained at each level throughout the incubation period. The same was true for MMI. On the other hand, the possibility that differences in the mitogen concentrations played a role cannot be excluded. In addition, it is not known whether the two PTU preparations employed were comparable; the present study used 6-propylthiouracil, while the PTU used in the preceding study appears to have been 5-propylthiouracil. In the report of Wall *et al.* (8), moreover, statistical considerations are lacking.

The current report extended the study of the effect of PTU to peripheral blood lymphocytes from hyperthyroid subjects. It was seen that the slight suppressive effect of PTU on PHA-induced activation of lymphocytes from normal subjects could be reproduced with lymphocytes from hyperthyroid patients.

The present investigation included experiments not only on PTU but also on MMI, and the effect of MMI was found to differ from that of PTU. Instead of suppressing the response to PHA and Con A, high concentrations of MMI enhanced PHA- and Con A-induced activation. On the other hand, PTU and MMI had similar, enhancing effects on PWM-induced activation. Thus, it seems that the two antithyroid agents differ in their immunomodulatory capacity. Moreover, the two drugs possibly interact differently with different mitogens. This is of interest since the mitogens partially activate different populations of lymphocytes; PHA and Con A have selective capacities to trigger T-lymphocytes into division, while PWM triggers both T- and, subsequently, B-lymphocytes into increased DNA synthesis (13).

As Graves' disease is probably an immunological disorder (1, 2), it is of interest if antithyroid drugs interfere with the immune system. However, caution is necessary in assessing the clinical importance of the recorded effects, as they were rather small for both drugs although statistically significant. In addition, they seemed to occur mainly at concentrations exceeding those obtained in blood samples of healthy volunteers after the administration of large single doses of these drugs; for PTU, 50–65 $\mu\text{mol/liter}$ were reported after 200 mg orally (14), and for

MMI, 5–25 $\mu\text{mol/liter}$ were reported after 60 mg orally (12). On the other hand, experiments in animals and humans indicate that antithyroid agents accumulate in thyroid tissue (15), and it is possible, therefore, that drug concentrations similar to those necessary to exert an effect on mitogenic activation of lymphocytes may be attained in thyroid tissue during treatment of hyperthyroidism.

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References

- Volpe R 1977 The role of autoimmunity in hypoenocrine and hyperendocrine function. *Ann Intern Med* 87:86
- Aoki N, Pimmamaneni KM, DeGroot LJ 1979 Studies on suppressor cell function in thyroid diseases. *J Clin Endocrinol Metab* 48:803
- DeGroot LJ, Nieponimszcze H 1977 Biosynthesis of thyroid hormone: basic and clinical aspects. *Metabolism* 26:665
- Marchant B, Lees FH, Alexander WD 1978 Antithyroid drugs. *Pharmacol Ther* [B] 3:305
- Beck JS, Young RJ, Simpson JG, Gray ES, Nicol AG, Pegg CAS, Michie W 1973 Lymphoid tissue in the thyroid gland and thymus of patients with primary thyrotoxicosis. *Br J Surg* 60:769
- Pinchera A, Libert P, Martino E, Fenu GF, Grasso L, Rovis L, Baschieri L, Doria G 1969 Effects of antithyroid therapy on the long-acting thyroid stimulator and the antithyroglobulin antibodies. *J Clin Endocrinol Metab* 29:231
- DeGroot LJ 1977 Short-term antithyroid drug therapy. *N Engl J Med* 297:212
- Wall JR, Manwar GL, Greenwood DM, Walters BA 1976 The *in vitro* suppression of lectin induced ^3H -thymidine incorporation into DNA of peripheral blood lymphocytes after the addition of propylthiouracil. *J Clin Endocrinol Metab* 43:1406
- Bovum A 1968 Isolation of leukocytes from human blood. *Scand J Clin Lab Invest* [Suppl] 97:1219
- Skellern CG, Stenlake JB, Williams WD, McLarty DG 1974 Plasma concentrations of methimazole, a metabolite of carbimazole, in hyperthyroid patients. *Br J Clin Pharmacol* 1:265
- Skellern CG, Knight BL, Stenlake JB 1976 Improved method for the determination of methimazole in plasma by high-performance liquid chromatography. *J Chromatogr* 124:405
- Melander A, Hallengren B, Rosendahl-Helgesen S, Sjöberg, A-K, Wahlén-Boll E 1980 Comparative effects and kinetics of antithyroid drugs. *Eur J Clin Pharmacol* 17:295
- Forsgren A, Svedjelund A, Wiggzell H 1976 Protein A from *Staphylococcus* as a human lymphocyte mitogen. In: Bloom BR (ed) *In Vitro Methods in Cell-Mediated and Tumor Immunity*. Academic Press, New York, p 193
- Sitar DS, Hunninghake DB 1975 Pharmacokinetics of propylthiouracil in man after a single oral dose. *J Clin Endocrinol Metab* 40:26
- Marchant B, Alexander WD, Lazarus JH, Lees J, Clark DM 1972 The accumulation of ^{125}I -antithyroid drugs by the thyroid gland. *J Clin Endocrinol Metab* 34:847

INFLUENCE OF β -ADRENOCEPTOR BLOCKING AGENTS ON LYMPHOCYTE FUNCTION IN VITRO

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1 In order to assess whether β -adrenoceptor blocking agents may have an immunomodulatory influence, the *in vitro* effect of propranolol, metoprolol and atenolol on mitogenic activation of human peripheral blood lymphocytes was studied. To investigate further the mechanism of this influence, the effects of quinidine and two other membrane stabilizing agents were examined.

2 At 100 and 1000 $\mu\text{mol/l}$, both D-propranolol, L-propranolol and D-, L-propranolol evoked a strong reduction of mitogenic lymphocyte activation. A similar effect was obtained with quinidine. Even at 1000 $\mu\text{mol/l}$, metoprolol had only a slight suppressive effect and atenolol had no suppressive influence at all.

3 It appears that propranolol may suppress mitogenic activation of lymphocytes, and that the effect may be ascribed to membrane stabilization rather than to β -adrenoceptor blockade.

INTRODUCTION

β -adrenoceptor blocking agents are increasingly used in the treatment of Graves' disease. As this malady seems to be a consequence of an immunological disturbance (Kidd et al., 1980) it is of interest whether β -adrenoceptor blockers have immunomodulatory properties. Such a possibility is suggested by animal studies showing that propranolol enhances antibody production in mice (Nakazawa et al., 1976) and rats (Benner et al., 1968). In addition, propranolol can inhibit mitogenic activation of human lymphocytes (Hadden et al., 1970). Recently, propranolol has been found to influence the function of neutrophilic leucocytes (Anderson & van Rensburg, 1979). To obtain further information on these aspects, the present study assessed the influence of different β -adrenoceptor blockers on mitogenic activation of human lymphocytes. In addition, a comparison was made with some membrane-stabilizing agents.

METHODS

Lymphocyte sampling

Lymphocytes were obtained from heparinized venous blood samples (40 ml) of clinically healthy, laboratory subjects ($n = 21$). The cells were prepared by centrifugation on Ficoll-Isopaque (Lymphoprep, Nyegaard & Co A/S, Oslo, Norway) as described by Bøyum (1968).

Lymphocyte culture

Lymphocyte samples were cultured at 37°C in microtiter plates (Linbro Scientific Inc., Hamden, CT, USA) containing 2.5×10^5 lymphocytes/culture in 200 μ l of serum-free RPMI (Gibco-Bio-Cult, Glasgow, Scotland) with the addition of gentamicin (12 μ g/ml), glutamine (2 mmol/l) and 1 g (5 g when testing lignocaine chloride, procaine hydrochloride and quinidine sulphate) bovine serum albumin per litre. The lymphocytes were incubated in the presence of phytohaemagglutinin (PHA; Wellcome Ltd, London, England, 1.25 μ g/ml) or pokeweed mitogen (PWM; Sigma Chemical Co., St Louis, MO, USA, 2.5 μ g/ml). The concentrations were chosen to give optimal lymphocyte stimulation.

Measurements of DNA synthesis

[³H]-thymidine (0.5 µCi; NET-027Z, SA 48 Ci/mmol, New England Nuclear Corp., Boston, MA, USA) in RPMI 1640 was added after 48 h of culture, *i.e.* 22-24 h before termination of cultures. The cells were harvested on glass fibre filters and rinsed in distilled water using a Skatron harvesting machine (Lierbyen, Oslo, Norway). The filters were dried 30 min under a heat lamp and transferred to scintillation vials containing 4 ml Insta-Fluor solution (Packard, Downers Grove, IL, USA). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter.

All cultures were carried out in triplicate except controls which were in quintuplicate. The median value of each triplicate was calculated as the percentage of control, *i.e.* the value derived from culture with mitogen in the absence of drugs, and are shown together with s.e. mean.

Drugs

D-Propranolol (hydrochloride), L-propranolol (hydrochloride), D-, L-propranolol (hydrochloride) and atenolol were supplied by Imperial Chemical Industries Ltd, Macclesfield, England, metoprolol (tartrate), quinidine (sulphate) and procaine (hydrochloride) by AB Hässle, Mölndal, Sweden, and lignocaine (hydrochloride) by AB Astra, Södertälje, Sweden. The drugs were dissolved and diluted in RPMI 1640 and were added to cultures simultaneously with mitogens from the beginning of the culture. All drugs were tested in the following final concentrations: 0.01, 0.1, 1, 10, 100 and 1000 µmol/l.

Measurements of drug concentrations

In order to check whether the desired concentrations of β-adrenoceptor blockers in incubation media were reached and maintained, their post-incubation concentrations were measured by high-pressure liquid chromatography (Yee et al., 1979; Scales & Copsey, 1975; Pritchard et al., 1979).

Cell viability

Cell viability was determined by trypan blue dye exclusion.

Statistics

The statistical significance of differences was calculated by Student's *t*-test.

RESULTS

Figure 1 shows the influence of D-propranolol, L-propranolol and D-, L-propranolol on PHA-activation of peripheral blood lymphocytes. At 100 and 1000 $\mu\text{mol/l}$, all propranolol forms gave a strong reduction ($p < 0.001$) of PHA-induced accumulation of [^3H]-thymidine. With L-propranolol a minor reduction was seen at 10 $\mu\text{mol/l}$ ($p < 0.05$), whereas a slight augmentation was recorded at 0.1 $\mu\text{mol/l}$ ($p < 0.05$).

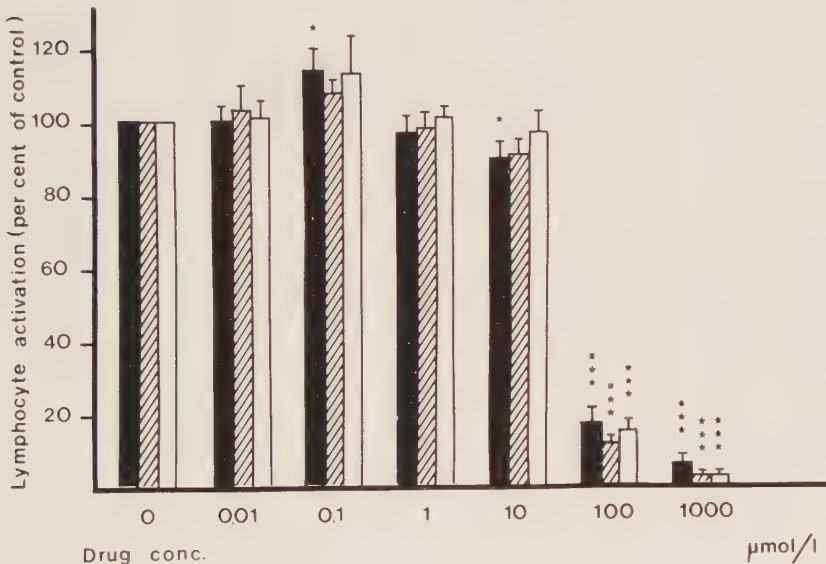


Figure 1 Uptake of [^3H]-thymidine in normal human peripheral blood lymphocytes activated with phytohaemagglutinin (PHA) 1.25 $\mu\text{g/ml}$ in the presence and absence of L-propranolol (■), D-, L-propranolol (▨) or D-propranolol (□) (mean + s.e. mean), $n = 7$.

* $p < 0.05$, *** $p < 0.001$.

Neither D- nor D-, L-propranolol had statistically significant effects at these concentrations. In a small series ($n = 3$) of experiments using drug concentrations between 10 and 100 $\mu\text{mol/l}$, D-propranolol evoked a mean reduction of 37%, L-propranolol a mean reduction of 25% and D-, L-propranolol a mean reduction of 39% at 25 $\mu\text{mol/l}$. The corresponding mean reductions at 50 $\mu\text{mol/l}$ were 55%, 44% and 55%, respectively. With PWM as mitogen, D-, L-propranolol gave a strong reduction ($p < 0.001$) at 100 and 1000 $\mu\text{mol/l}$ (Figure 2).

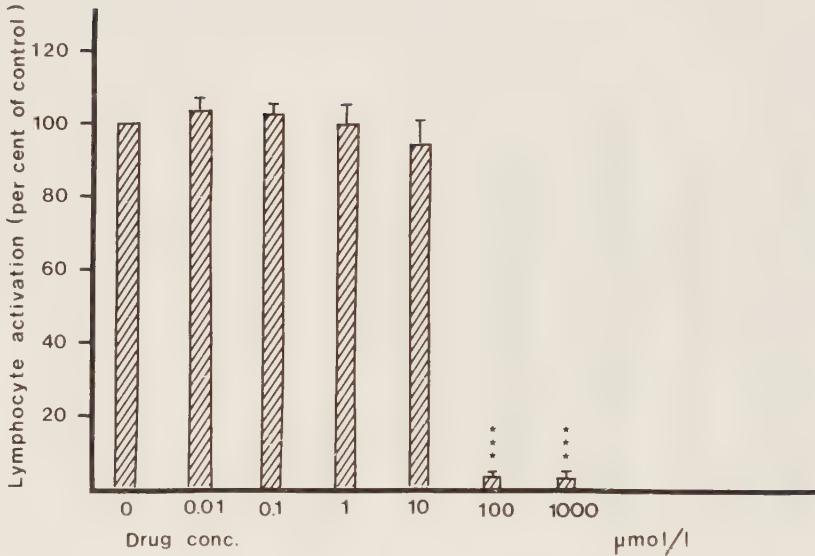


Figure 2 Uptake of [^3H]-thymidine in normal peripheral blood lymphocytes activated with pokeweed mitogen (PWM) 2.5 $\mu\text{g/ml}$ in the presence and absence of D-, L-propranolol (mean + s.e. mean), $n = 8$.

*** $p < 0.001$.

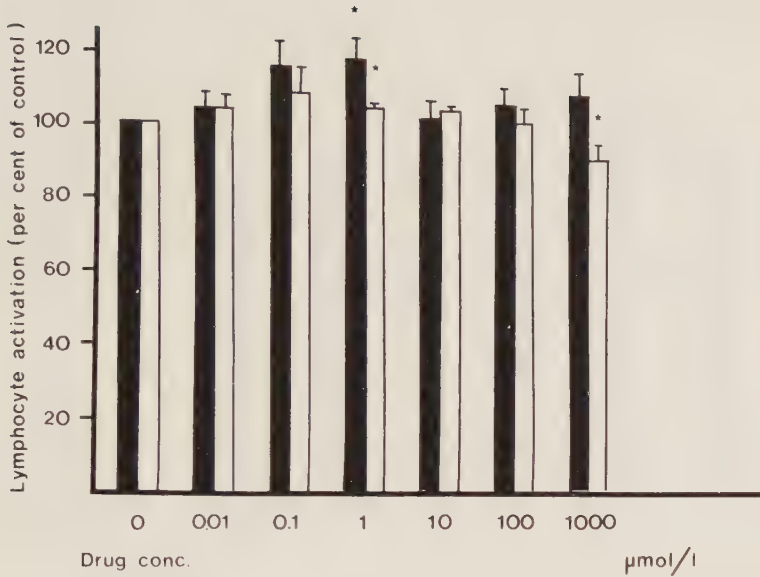


Figure 3 Uptake of [^3H]-thymidine in normal human peripheral blood lymphocytes activated with phytohaemagglutinin (PHA) 1.25 $\mu\text{g}/\text{ml}$ in the presence and absence of atenolol (■) or metoprolol (□) (mean + s.e. mean), $n = 7$.

* $p < 0.05$.

Figure 3 demonstrates the influence of metoprolol and atenolol. At 1000 $\mu\text{mol}/\text{l}$ there was a slight reduction ($p < 0.05$) with metoprolol and at 1 $\mu\text{mol}/\text{l}$ a minor augmentation ($p < 0.02$) of PHA-induced accumulation of [^3H]-thymidine. Atenolol did not significantly reduce PHA-induced [^3H]-thymidine incorporation at any concentration used; instead there was a slight but significant ($p < 0.02$) augmentation at 1 $\mu\text{mol}/\text{l}$. With PWM as mitogen atenolol had no significant effect ($n = 9$).

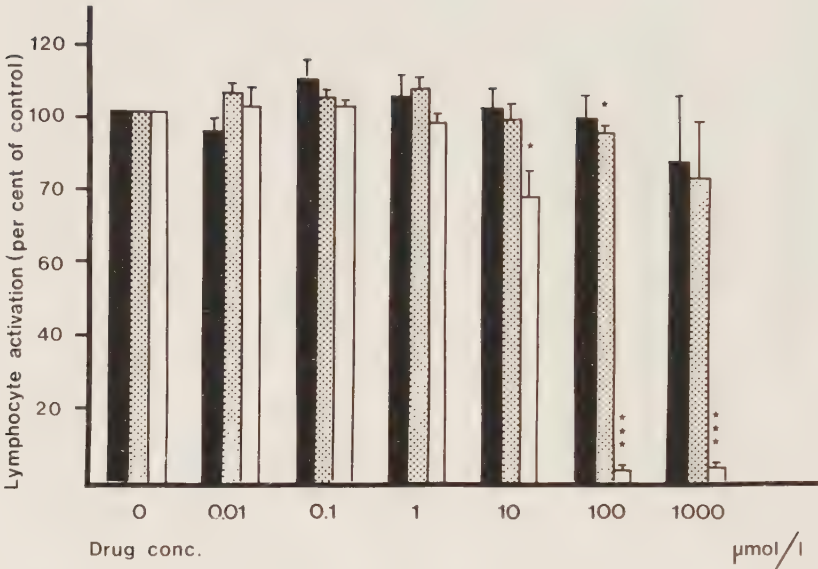


Figure 4 Uptake of [^3H]-thymidine in normal human peripheral blood lymphocytes activated with phytohaemagglutinin (PHA) 1.25 $\mu\text{g}/\text{ml}$ in the presence and absence of procaine (■, $n = 5$), lignocaine (▨, $n = 7$) or quinidine (□, $n = 5$) (mean + s.e. mean).

* $p < 0.05$, *** $p < 0.001$.

Figure 4 shows that lignocaine had a slight inhibitory influence on [^3H]-thymidine incorporation at 100 $\mu\text{mol}/\text{l}$ ($p < 0.05$) and no statistically significant influence at other concentrations. Procaine at the same concentrations as lignocaine had no significant effect ($n = 5$). In contrast, quinidine at 100 and 1000 $\mu\text{mol}/\text{l}$ strongly reduced ($p < 0.001$) PHA-induced accumulation of [^3H]-thymidine and at 10 $\mu\text{mol}/\text{l}$ evoked a 23% reduction ($p < 0.05$).

Cell viability

After 72 h of culture the following results of cell viability were found: RPMI without drug 91%, metoprolol 1000 $\mu\text{mol/l}$ 90%, D-propranolol 1000 $\mu\text{mol/l}$ 0%, 100 $\mu\text{mol/l}$ 91%, L-propranolol 1000 $\mu\text{mol/l}$ 1%, 100 $\mu\text{mol/l}$ 85%, D-, L-propranolol 1000 $\mu\text{mol/l}$ 1%, 100 $\mu\text{mol/l}$ 91%.

Drug concentrations

Measurements of the concentrations of propranolol, metoprolol and atenolol in the incubation media before and after 72 h incubation revealed no degradation of either β -adrenoceptor blocker.

DISCUSSION

The present investigation indicates that, at the lower tested concentrations, both propranolol, metoprolol and atenolol may increase PHA-activation of lymphocytes. The stimulatory effects were, however, very slight and therefore of doubtful clinical relevance. At higher (100-1000 $\mu\text{mol/l}$) concentrations of propranolol, but not of the other two β -adrenoceptor blockers, both PHA- and PWM-induced activation were strongly reduced instead. At the highest (1000 $\mu\text{mol/l}$) propranolol concentration, lymphocyte viability was impaired.

The inhibitory effect of propranolol at the higher tested concentrations agrees with a previous report by Hadden et al. (1970). While D-propranolol, in contrast to L-propranolol, is virtually devoid of β -adrenoceptor blocking capacity, both isomers have similar membrane-stabilizing or quinidine-like properties (Waal-Manning, 1976). At 100 $\mu\text{mol/l}$, D-propranolol and also quinidine seemed as effective as L-propranolol. This indicates that the recorded inhibitory effect of propranolol may be due to a membrane-stabilizing effect rather than to genuine β -adrenoceptor blockade. This view is further supported by the observation that the cardioselective β -adrenoceptor blocker metoprolol, which has very weak membrane-stabilizing properties (Waal-Manning, 1976), exhibited only a slight suppressive effect at the highest dose (1000 $\mu\text{mol/l}$). In addition, atenolol, which is devoid of membrane-stabilizing properties (Waal-Manning, 1976), had no suppressive influence at any concentration used. It is of interest in this context that D-propranolol, like D-, L-propranolol, is capable of inhibiting conversion of T_4 to T_3 , and thus this inhibitory effect may be due to

membrane stabilization rather than β -adrenoceptor blockade (Heyma et al., 1980).

As for the clinical significance of the employed concentrations it may be noted that, during propranolol (320 mg daily) treatment of hyperthyroid patients, the drug concentrations in plasma were less than 1 $\mu\text{mol/l}$ (Nilsson et al., 1980). In the present study the pronounced *in vitro* effects with propranolol were seen only at higher concentrations. It is difficult, however, to compare *in vivo* and *in vitro* concentrations since *in vitro* systems may have a reduced sensitivity due to absence of *in vivo* factors (Segal & Ingbar, 1980).

Procaine did not measurably inhibit lymphocyte stimulation. This agrees with the fact that the local anaesthetic activity of procaine is less than that of propranolol (Dollery et al., 1969). The slight effect of lignocaine at the tested concentrations is in agreement with observations by Cullen et al. (1972) and by Ferguson et al. (1976); the latter recorded a pronounced suppressive effect of lignocaine only at a very high concentration (2000 $\mu\text{mol/l}$).

In conclusion, it appears that propranolol may suppress mitogenic activation of human peripheral blood lymphocytes, and that this effect may be ascribed to membrane stabilization rather than to β -adrenoceptor blockade.

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REFERENCES

- Anderson, R. & van Rensburg, A.J. (1979). The *in vitro* effects of propranolol and atenolol on neutrophil motility and post-phagocytic metabolic activity. *Immunology*, 37, 15-23.
- Benner, M.H., Enta, T., Lockey, S., Makino, S. & Reed, C.E. (1968). The immuno-suppressive effect of epinephrine and the adjuvant effect of beta-adrenergic blockade. *J. Allergy*, 41, 110-111.
- Bøyum, A. (1968) Isolation of leucocytes from human blood. *Scand J. clin. lab. Invest. (Suppl 97)* 21, 9-89.
- Cullen, B.F., Chretien, P.B. & Leventhal, B.G. (1972). The effect of lignocaine on PHA-stimulated human lymphocyte transformation. *Br. J. Anaesth.*, 44, 1247-1252.
- Dollery, C.T., Paterson, J.W. & Conolly, M.E. (1969). Clinical pharmacology of beta-receptor-blocking drugs. *Clin. Pharmac. Ther.*, 10, 765-799.
- Ferguson, R.M., Schmidtke, J.R. & Simmons, R.L. (1976). Inhibition of mitogen-induced lymphocyte transformation by local anaesthetics. *J. Immun.*, 116, 627-634.
- Hadden, J.W., Hadden, E.M. & Middleton, Jr., E. (1970). Lymphocyte blast transformation I. Demonstration of adrenergic receptors in human peripheral lymphocytes. *Cell Immun.*, 1, 583-595.
- Heyma, P., Larkins, R.G., Higginbotham, L. & Wahng, K. (1980). D-propranolol and DL-propranolol both decrease conversion of L-thyroxine to L-triiodothyronine. *Br. med. J.*, 281, 24-25.
- Kidd, A., Okita, N., Row, V.V. & Volpé, R. (1980). Immunologic aspects of Graves' and Hashimoto's diseases. *Metabolism*, 29, 80-89.
- Nakazawa, H., Adolphson, R., Chaperon, E., Hobday, J., Townley, R. & Pauli, J. (1976). *In vivo* and *in vitro* effects of chronic propranolol administration on the immune response in mice. *J. Allergy clin. Immun.*, 57, 200.
- Nilsson, O.R., Melander, A. & Tegler, L. (1980). Effects and plasma levels of propranolol and metoprolol in hyperthyroid patients. *Eur. J. clin. Pharmac.*, 18, 315-320.
- Pritchard, J.F., Schneck, D.W. & Hayes, Jr., A.H. (1979). Determination of propranolol and six metabolites in human urine by high-pressure liquid chromatography. *J. Chromatogr.*, 162, 47-58.
- Scales, B. & Copsey, P.B. (1975). The gas chromatographic determination of atenolol in biological samples. *J. Pharm. Pharmac.*, 27, 430-433.

- Segal, J. & Ingbar, S.H. (1980). Stimulation of 2-deoxy-D-glucose uptake in rat thymocytes *in vitro* by physiological concentrations of triiodothyronine, insulin or epinephrine. *Endocrinology* 107, 1354-1358.
- Waal-Manning, H.J. (1976). Hypertension: Which beta-blocker? *Drugs*, 12, 412-441.
- Yee, Y.-G., Rubin, P. & Blaschke, T.F. (1979). Atenolol determination by high-performance liquid chromatography and fluorescence detection. *J. Chromatogr.*, 171, 357-362.

Influence of Hyperthyroidism on the Kinetics of Methimazole, Propranolol, Metoprolol and Atenolol

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Summary. The kinetic profiles of oral methimazole 40 mg, propranolol 80 mg, metoprolol 100 mg and atenolol 100 mg were compared in hyperthyroid patients both during the hyper- and euthyroid states. For methimazole, neither the peak concentration (C_{max}), the time to reach peak concentration (t_{max}), the elimination half-life ($t_{1/2}$) nor the area under the curve (AUC) value was affected by the hyperthyroid state. For propranolol and metoprolol, which undergo extensive presystemic clearance, the AUC values were lower ($p < 0.02$) when the patients were hyperthyroid than when they had become euthyroid, but the $t_{1/2}$'s were not significantly altered. For atenolol, there were no significant kinetic differences between the hyperthyroid and euthyroid states. The findings are compatible with the assumption that hyperthyroidism does not affect the kinetics of methimazole or atenolol, but that it may enhance presystemic clearance of propranolol and metoprolol.

Key words: hyperthyroidism, propranolol, methimazole; metoprolol, atenolol, kinetics

Thionamides, such as methimazole, and β -adrenoceptor blocking agents (β -blockers), such as propranolol, are commonly used in the pharmacotherapy of hyperthyroidism. Methimazole inhibits thyroid hormone biosynthesis [1] and may have an immunomodulatory influence [2], while β -blockers seem to inhibit certain effects of thyroid hormones [3].

There is great interindividual variation in the kinetics both of methimazole [4, 5] and propranolol [6]. In addition, it is possible that the handling of methimazole and propranolol may be influenced by the hyperthyroid state and its subsequent regression follow-

ing therapy. Apart from propranolol, atenolol [7] and metoprolol [3] have been successfully used in hyperthyroid patients. As propranolol and metoprolol are extensively metabolized, whereas atenolol is excreted unchanged [6], hyperthyroidism may affect the kinetics of these agents differently.

The present study is a comparison of the kinetic profiles of oral methimazole, propranolol, metoprolol and atenolol in hyperthyroid patients during the hyper- and euthyroid states.

Patients and Methods

Patients and Medication

29 patients with hyperthyroidism (Graves' disease or toxic multinodular goitre) were studied after their informed consent had been obtained. Examinations were carried out both in the hyperthyroid and the euthyroid state. The patients in Malmö ($n = 8$) received methimazole, while the patients in Linköping ($n = 21$) received propranolol ($n = 7$), metoprolol ($n = 7$) or atenolol ($n = 7$). The diagnosis of hyperthyroidism was based on clinical examination and on determination of serum triiodothyronine (T_3) and thyroxine (T_4 ; Table 1). Serum T_3 concentrations were measured by radioimmunoassay [8, 9]. Serum T_4 concentrations were measured by radioimmunoassay ($n = 8$; [8]), or by a competitive protein binding method ($n = 21$; [10]). After fasting overnight, each patient received a single oral dose of methimazole¹ 40 mg, propranolol² 80 mg, metoprolol³ 100 mg or atenolol⁴ 100 mg. Meth-

¹ Thacapzol®, KABI, Stockholm, Sweden

² Inderal®, ICI Pharmaceuticals, Macclesfield, England

³ Seloken®, Hässle (subsidiary of Astra), Mölndal, Sweden

⁴ Tenormin®, ICI Pharmaceuticals, Macclesfield, England

Table 1. Clinical and biochemical characteristics of the groups of patients (mean ± SEM)

Group	Sex	Age [years]	Hyperthyroidism			Euthyroidism		
			Heart rate [beats/min]	Triiodo- thyronine [nmol/l]	Thyroxine [nmol/l]	Heart rate [beats/min]	Triiodo- thyronine [nmol/l]	Thyroxine [nmol/l]
Methimazole (n = 8)	5F, 3M	47 ± 5	98 ± 4	6.3 ± 1.3	229 ± 9	76 ± 4	1.8 ± 0.3 ^a	95 ± 7 ^c
Propranolol (n = 7)	5F, 2M	40 ± 7	99 ± 5	7.6 ± 1.2	243 ± 20	66 ± 2	1.9 ± 0.1 ^b	114 ± 10 ^d
Metoprolol (n = 7)	6F, 1M	51 ± 4	96 ± 6	5.5 ± 0.7	207 ± 9	65 ± 4	1.7 ± 0.1 ^b	98 ± 9 ^d
Atenolol (n = 7)	6F, 1M	54 ± 5	99 ± 4	7.2 ± 1.3	208 ± 21	73 ± 4	1.8 ± 0.1 ^b	95 ± 9 ^d

^a Reference range 0.9–3.2

^b Reference range 1.2–2.5

^c Reference range 50–150

^d Reference range 60–140

Table 2. Therapy to achieve euthyroidism in the 29 hyperthyroid patients

Group	Thyroid resection	Thionamides	Radioiodine
Methimazole (n = 8)		8	3
Propranolol (n = 7)	4	2	1
Metoprolol (n = 7)	4	1	2
Atenolol (n = 7)	3	1	3

imazole was taken 2 h before breakfast, and the β -blockers were ingested immediately before a standardized breakfast of 1840 kJ [11]. The same drugs were again administered to the same patients in the identical manner, after clinical and biochemical euthyroidism had been maintained for at least 4 weeks, either by medication or following surgery or radioiodine treatment, sometimes combined with thionamides (Table 2). In patients rendered euthyroid on thionamides, this medication was withdrawn 18–24 h before the second study. Six of the patients tested with methimazole had been given a β -blocker, which was withdrawn at least 12 h before examination. One of the patients tested with methimazole was on continuous treatment with furosemide and potassium chloride because of previous cardiac failure, and another patient was on nitrazepam. Two patients tested with atenolol had had digoxin treatment for many years; they had no signs of cardiac failure at the time of the study. One female given propranolol was on continuous oral contraception.

Blood Sampling and Drug Analyses

Blood samples for analyses of drug concentrations in plasma were taken before and repeatedly after drug administration. Methimazole concentrations were always measured within 24 h of sampling [cf. 4]. As the β -blocker samples were obtained in Linköping but analyzed in Malmö, whether storage at -20°C affected the concentrations of propranolol, metoprolol or atenolol was examined. To this end, plasma samples with known amounts of either drug were analyzed after 0, 1, 2, 3 and 6 months of such storage. There was no indication of degradation of any of the β -blockers during storage. Accordingly, all samples from the same individual were analyzed in one assay, minimizing interassay variation.

The plasma concentrations of methimazole [12], propranolol [13] and atenolol [14] were determined by high-pressure liquid chromatography, and those of metoprolol by gas chromatography [15].

Calculations

The elimination half-life of each drug ($t_{1/2}$) was calculated by regression analysis. The area under the concentration curve (AUC) was determined by the trapezoidal rule. The residual ($8\text{ h} \rightarrow \infty$) AUC values ("rest areas") of the β -blockers were not included in the AUC calculations, as they were rather large and so were difficult to estimate accurately. Peak concentration (C_{max}) was defined as the highest value actually recorded, and the time to reach peak concentration (t_{max}) was obtained accordingly. As none of the three β -blockers has 100% bioavailability [6], and as the bioavailability of methimazole is unknown, calculation of absorption and distribution kinetics was not

Table 3a. Individual kinetic estimates of **a** methimazole (*n* = 8), **b** propranolol (*n* = 7), **c** metoprolol (*n* = 7) and **d** atenolol (*n* = 7) in hyperthyroid patients before and after treatment-induced euthyroidism. *C*_{max} = peak concentration, *t*_{max} = time to reach peak concentration, *t*_{1/2} = elimination half-life, AUC = area under the concentration curve. HT = hyperthyroid state, ET = euthyroid state, N.S. = not significant

Patient no.	<i>Methimazole</i>		∞										Time between examinations [weeks]
	C_{max}		t_{max}		$t_{1/2}$		AUC $0 \rightarrow \infty$ h		T_3		Body weight		
	[μmol/l]		[h]		[h]		[μmol × h × l ⁻¹]		[nmol/l]		[kg]		
	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET	
1	8.85	5.69	0.5	2.0	2.9	4.2	38.76	43.92	5.8	1.7	52	54	5
2	9.46	6.13	0.25	0.75	3.6	6.8	28.50	33.29	3.5	1.5	85	90	5
3	5.74	8.41	1.0	0.75	3.8	5.0	35.04	43.48	12.0	3.9	69	73	13
4	4.56	7.98	0.75	0.50	5.0	3.5	37.55	29.13	3.6	1.3	77	83	6
5	8.41	6.57	0.90	0.75	4.4	6.0	68.92	66.30	> 12	1.2	74	78	10
6	7.10	9.46	1.0	0.50	7.1	7.3	80.29	86.59	4.4	1.0	83	83	8
7	5.39	5.93	3.0	2.0	3.8	4.4	42.69	45.76	4.9	1.5	50	51	6
8	8.76	7.62	0.75	1.0	3.6	3.1	51.21	41.89	4.4	2.1	55	57	13
	N.S.		N.S.		N.S.		N.S.						

Table 3b

Patient no.	Propranolol													Time between examinations [months]
	C _{max}		t _{max}		t _{1/2}		AUC 0→8 h		T ₃		Body weight			
	[nmol/l]		[h]		[h]		[nmol × h × l ⁻¹]		[nmol/l]		[kg]			
	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET		
9	413	567	1.0	2.0	2.8	3.5	1903	2772	7.9	1.5	66	69	2	
10	520	355	0.5	2.0	2.0	2.0	1024	1587	10.0	2.4	56	50	5	
11	93	220	1.0	1.5	3.1	6.4	316	926	6.7	1.7	68	77	6	
12	513	470	1.5	2.0	6.3	5.5	1998	2733	3.1	2.4	51	54	12	
13	147	208	1.0	1.0	4.6	2.4	591	720	12.9	1.7	53	56	4	
14	316	316	0.5	2.0	7.7	3.4	1430	1491	8.3	2.2	71	71	6	
15	470	678	1.5	1.5	3.8	3.6	1494	2901	4.6	1.6	63	70	20	
	N.S.		N.S.		N.S.		p < 0.02 (mean increase: 64%)							

Table 3c

Patient no.	Metoprolol														Time between examinations [months]
	C _{max}		t _{max}		t _{1/2}		AUC 0 → 8 h		T ₃		Body weight				
	[nmol/l]		[h]		[h]		[nmol × h × l ⁻¹]		[nmol/l]		[kg]				
	HT	ET	HT	ET	HT	ET	HT	EWT	HT	ET	HT	ET			
16	970	920	0.5	2.0	7.0	6.8	5369	5452	4.3	2.1	67	70	2		
17	883	785	0.5	0.5	2.3	6.9	2173	3768	4.9	1.3	81	87	5		
18	430	596	1.0	1.5	2.4	3.0	1590	2555	8.9	2.1	74	77	5		
19	390	398	1.0	1.5	1.7	1.9	1086	1330	3.5	1.3	69	73	8		
20	460	484	0.5	1.5	1.8	2.3	1126	2088	6.7	1.3	49	52	6		
21	510	641	1.0	1.5	2.5	3.8	1936	2979	3.8	1.7	72	75	6		
22	337	507	1.0	1.5	3.3	3.0	1457	2585	6.2	2.0	50	63	3		
	N.S.		p < 0.05 (mean diff.: 0.65 h)		N.S.		P < 0.02 (mean increase: 53%)								

Table 3d

Patient no.	Atenolol														Time between examinations [months]
	C_{max}		t_{max}		$t_{1/2}$		AUC 0→8 h		T_3		Body weight				
	[μmol/l]		[h]		[h]		[μmol × h × l ⁻¹]		[nmol/l]		[kg]				
	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET	HT	ET			
23	3.23	2.93	1.5	1.5	2.2	2.95	16.16	12.35	8.4	1.9	52	60	5		
24	1.05	1.73	1.5	3.0	8.1	6.5	5.95	9.70	3.0	1.7	69	72	6		
25	4.24	3.90	2.0	2.0	4.45	8.3	21.15	22.93	4.8	1.8	48	49	6		
26	1.65	2.66	3.0	2.0	3.3	5.35	7.71	14.83	5.1	1.7	71	72	7		
27	2.14	3.04	2.0	1.5	3.55	3.8	11.11	13.56	13.7	2.3	61	63	6		
28	1.58	4.58	3.0	2.0	4.3	2.2	8.20	18.57	7.6	1.8	72	75	5		
29	1.58	2.06	3.0	1.5	4.2	3.25	10.96	8.38	7.6	1.5	78	87	18		
	N.S.		N.S.		N.S.		N.S.								

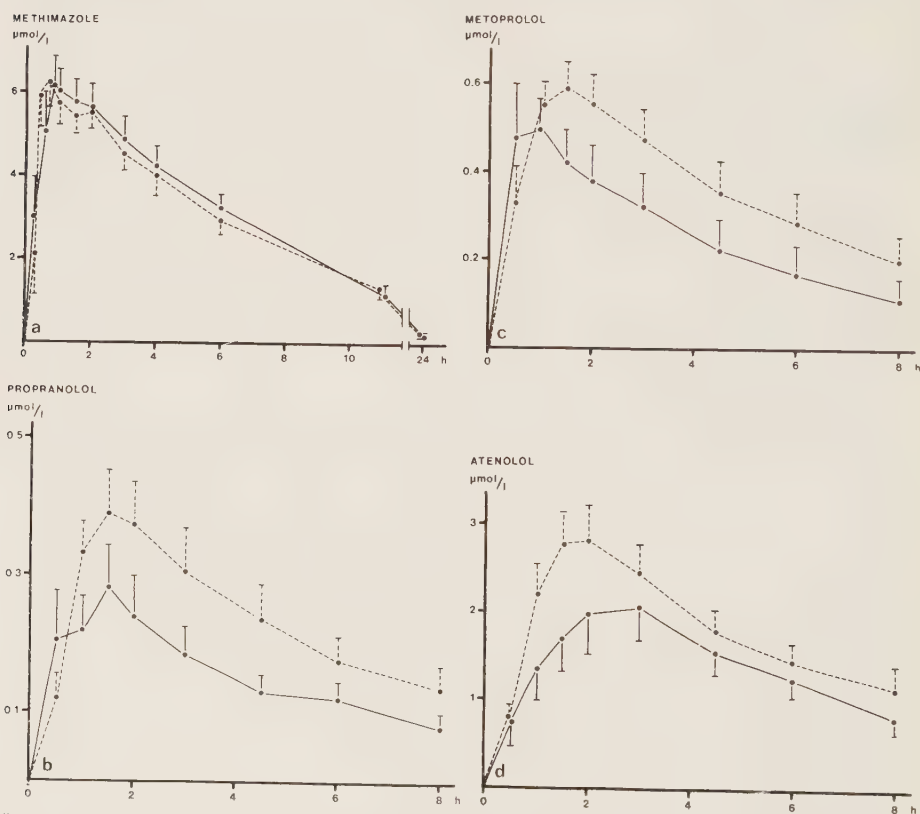


Fig. 1a-d. Kinetic profiles (mean $\pm$ SEM) of orally administered **a** methimazole ($n = 8$), **b** propranolol ($n = 7$), **c** metoprolol ($n = 7$) and **d** atenolol ($n = 7$) in patients during the hyper- (—) and euthyroid (---) states

justified. Wilcoxon's rank sum test for paired samples was used to determine the statistical significance of intra-individual differences.

Results

The individual kinetic values are shown in Table 3a–d, and the mean kinetic profiles are shown in Fig. 1a–d. It can be seen that, for methimazole, neither the $C_{\max}$, the $t_{\max}$, the $t_{1/2}$, nor the AUC ($0\text{ h} \rightarrow \infty$) values were affected by the hyperthyroid state.

The AUC (0–8 h) values both for propranolol and metoprolol, on the other hand, were lower ($p < 0.02$) in hyperthyroidism than when the patients had become euthyroid. As the $t_{\max}$ for propranolol and metoprolol were shortened rather than prolonged during hyperthyroidism, the cut-off of the AUC at 8 h would under- rather than overestimate the true difference. Hence, the calculated difference in the AUCs most probably reflects a true difference. The $C_{\max}$ and the $t_{1/2}$ for propranolol and metoprolol were not significantly different.

In contrast to those of propranolol and metoprolol, the AUC (0–8 h) values of atenolol were not significantly different between the hyperthyroid and the euthyroid states; nor were the $C_{\max}$, $t_{\max}$ and $t_{1/2}$ values significantly influenced.

Discussion

As hyperthyroidism increases the general metabolic rate, it would seem logical to suspect that the biotransformation of drugs would be enhanced in patients with this disorder. There are, however, few data to support this generalization [16], and it has been contradicted by findings with certain drugs [17, 18].

Both propranolol and metoprolol are subject to extensive presystemic clearance, probably by identical biochemical processes [6]. Hence, it seems likely that the reduced AUC of propranolol and metoprolol during the hyperthyroid state indicates enhanced presystemic clearance of these two β -blockers in hyperthyroidism. A diminished peak concentration might also be anticipated, but the $C_{\max}$ of both compounds was not significantly reduced. There was no significant change in $t_{1/2}$ either, but enhanced presystemic clearance of drugs can occur without affecting $t_{1/2}$ [19].

While the observations on metoprolol are novel, the findings for propranolol agree with those of Feely et al. [20]. Riddell et al. [21] observed a similar, albeit insignificant, tendency for propranolol. Moreover, Riddell et al. [21] and Rubinfeld et al. [22] showed that the systemic clearance of propranolol is increased fol-

lowing intravenous administration. Thus, it seems likely that hyperthyroidism may enhance both the presystemic and the systemic clearance of propranolol and metoprolol. An altered distribution volume, e.g. reduced binding, may contribute to this [20, 22], and hyperthyroidism has been found to affect the distribution volume of several other drugs, such as propylthiouracil [23] and paracetamol [24].

The present findings, and those of Feely et al. [20] and Riddell et al. [21] about propranolol, are in contrast to those of Theilade et al. [25], who reported an increased AUC of propranolol in hyperthyroid patients as compared to healthy volunteers. There is no obvious explanation for this discrepancy, but it may relate to the fact that the data from the present study and the two others [20, 21] were derived from within-patient comparison between hyperthyroidism and therapeutically achieved euthyroidism, whereas those of Theilade et al. [25] resulted from a between-subject comparison of hyperthyroid patients with healthy volunteers who had not suffered from hyperthyroidism.

In further agreement with the concept that hyperthyroidism would enhance the presystemic clearance of propranolol and metoprolol, it was found that hyperthyroidism affected neither the AUC nor the $t_{\max}$ of atenolol, a β -blocker which essentially escapes metabolic transformation [6].

The theory that hyperthyroidism would enhance drug metabolism in general is challenged by the finding that neither the AUC value nor any other kinetic parameter of methimazole was affected during the hyperthyroid state, even though methimazole undergoes rapid and extensive metabolism [26]. Moreover, its biotransformation seems to involve oxidation, like that of propranolol and metoprolol [26].

A possible explanation could be that the influence of hyperthyroidism on drug oxidation becomes apparent mainly for drugs that are subject to extensive presystemic clearance, as are propranolol and metoprolol [6]. It is not known whether methimazole undergoes first-pass metabolism, and little is known about its absolute bioavailability. Another possibility would be that propranolol/metoprolol and methimazole are metabolized by different oxidative systems, and that hyperthyroidism selectively enhances that involving the β -blockers. It should be mentioned in this context that altered handling of methimazole in hyperthyroidism has been reported [27]. However, this was based on observations with an unselective method incapable of separating methimazole from its metabolites [27].

Irrespective of the mechanisms involved, the results indicate that the kinetics of propranolol and metoprolol may be altered during the course of hyper-

thyroidism, whereas that of methimazole and atenolol remains essentially unchanged. Thus, there could be a pharmacokinetic basis for adjustment of the dose of the former two drugs, but not of the latter two compounds, during the course of hyperthyroidism. It must be emphasized, however, that the pharmacodynamics of all four drugs may be influenced by the change in thyroid activity, and that dose adjustment may also be warranted for that reason.

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References

- DeGroot LJ, Niepomniszcze H (1977) Biosynthesis of thyroid hormone: basic and clinical aspects. *Metabolism* 26: 665-718
- Hallengren B, Forsgren A, Melander A (1980) Effects of antithyroid drugs on lymphocyte function in vitro. *J Clin Endocrinol Metab* 51: 298-301
- Nilsson O, Melander A, Tegler L (1980) Effects and plasma levels of propranolol and metoprolol in hyperthyroid patients. *Eur J Clin Pharmacol* 18: 315-320
- Melander A, Hallengren B, Rosendal-Helgesen S, Sjöberg A-K, Wåhlin-Boll E (1980) Comparative in vitro effects and in vivo kinetics of antithyroid drugs. *Eur J Clin Pharmacol* 17: 295-299
- Skellern GG, Knight BI, Low CKL, Alexander WD, McLarty DG, Kalk WJ (1980) The pharmacokinetics of methimazole after oral administration of carbimazole and methimazole in hyperthyroid patients. *Br J Clin Pharmacol* 9: 137-143
- Johnsson G, Regårdh C-G (1976) Clinical Pharmacokinetics of β -adrenoceptor blocking drugs. *Clin Pharmacokinet* 1: 233-263
- Nilsson OR, Karlberg BE, Kågedal B, Tegler L, Almqvist S (1979) Non-selective and selective β_1 -adrenoceptor blocking agents in the treatment of hyperthyroidism. *Acta Med Scand* 206: 21-25
- Thorell JJ, Larson SA (1978) Radioimmunoassay and related techniques. CV Mosby, St. Louis, MO. p110
- Gharib H, Ryan RJ, Mayberry WE, Hockert T (1971) Radioimmunoassay for triiodothyronine (T_3): I Affinity and specificity of the antibody for T_3 . *J Clin Endocrinol Metab* 33: 509-516
- Seligson H, Seligson D (1972) Measurement of thyroxine by competitive protein binding. *Clin Chim Acta* 38: 199-205
- Melander A (1978) Influence of food on the bioavailability of drugs. *Clin Pharmacokinet* 3: 337-351
- Skellern GG, Stenlake JB, Williams WD, McLarty DG (1974) Plasma concentrations of methimazole, a metabolite of carbimazole, in hyperthyroid patients. *Br J Clin Pharmacol* 1: 265-269
- Pritchard JF, Schneck DW, Hayes Jr AH (1979) Determination of propranolol and six metabolites in human urine by high-pressure liquid chromatography. *J Chromatogr* 162: 47-58
- Yee Y-G, Rubin P, Blaschke TF (1979) Atenolol determination by high-performance liquid chromatography and fluorescence detection. *J Chromatogr* 171: 357-362
- Ervik M (1975) Quantitative determination of metoprolol in plasma and urine by gas chromatography. *Acta Pharmacol Toxicol [Suppl]* 36 (5): 136-144
- Eichelbaum M (1976) Drug metabolism in thyroid disease. *Clin Pharmacokinet* 1: 339-350
- Gillette JR (1965) Drug toxicity as a result of interference with physiological control mechanisms. *Ann NY Acad Sci* 123: 42-54
- Gordon GG, Southren AL, Tochimoto S, Rand JJ, Olivo J (1969) Effect of hyperthyroidism and hypothyroidism on the metabolism of testosterone and androstenedione in man. *J Clin Endocrinol* 29: 164-170
- Talseth T (1977) Clinical pharmacokinetics of hydralazine. *Clin Pharmacokinet* 2: 317-329
- Feely J, Crooks J, Stevenson IH (1979) Altered pharmacokinetics of propranolol in hyperthyroidism. *Br J Clin Pharmacol* 8: 387 P
- Riddell JG, Neill JD, Kelly JG, McDevitt DG (1980) Effects of thyroid dysfunction on propranolol kinetics. *Clin Pharmacol Ther* 28: 565-574
- Rubenfeld S, Silverman VE, Welch KMA, Mallette LE, Kohler PO (1978) Propranolol pharmacokinetics in thyrotoxicosis. *Clin Res* 26: 295 A
- Sitar DS, Abu-Bakare A, Gardiner RJ, Ogilvie RI (1979) Effect of chronic therapy with propylthiouracil on its disposition in hyperthyroid patients. *Clin Invest Med* 2: 93-97
- Forfar JC, Pottage A, Toft AD, Irvine WJ, Clements JA, Prescott LF (1980) Paracetamol pharmacokinetics in thyroid disease. *Eur J Clin Pharmacol* 18: 269-273
- Theilade P, Steiness E, Jørgensen PH, Hansen JM (1980) Decreased first-pass metabolism of propranolol in hyperthyroidism. *World Conf Clin Pharmacol Ther*, London, Abstract no.0608. Mac Millan, London
- Marchant B, Lees FH, Alexander WD (1978) Antithyroid drugs. *Pharmacol Ther [B]* 3: 305-348
- Vesell ES, Shapiro JR, Passananti GT, Jørgensen H, Shively CA (1975) Altered plasma half-lives of antipyrine, propylthiouracil, and methimazole in thyroid dysfunction. *Clin Pharmacol Ther* 17: 48-56

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